IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

ATTY.'S DOCKET: WALLACH=5B

In re Application of:	Conf. No.: 5293
David WALLACH et al	Art Unit: 1644
Appln. No.: 08/485,129	Examiner: R. Schwadron
Filed: June 7, 1995	Washington, D.C.
For: ISOLATED DNA ENCODING TUMOR NECROSIS FACTOR BINDING PROTEIN II, AND VECTORS, HOSTS AND PROCESSES USING SUCH DNA	February 4, 2002

REPLY BRIEF

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

Pursuant to 37 C.F.R. §1.193(b)(1), submitted herewith is appellant's reply brief in triplicate. Consideration of the present reply brief in conjunction with appellant's main brief on appeal is respectfully urged.

The Present Specification Contains Sufficient Disclosure To Establish that the Present Inventors Were in Possession of the Presently Claimed DNA

In the Examiner's Answer, the examiner's response to appellant's argument confuses the issue by repeatedly arguing that a nucleic acid sequence is not an inherent property of a protein. However, appellant does not take the position that written description of the DNA exists merely because the protein is adequately described. The syllogism of appellant's main brief must be considered in conjunction with the

disclosure of applicants' specification as originally filed. Thus, it is not mere attorney argument or sophistry which establishes the conclusion that those in possession of an amino acid sequence are also in possession of the genus of nucleic acids which encode that amino acid sequence. In this case, the present specification as filed described the claimed DNA in sufficient detail to establish that applicants were in possession of the genus of DNA, each species of which encode TBP-II. In this regard, reference is made to page 8, lines 20-23, of the present specification where it states:

> Thus the invention concerns DNA molecules comprising the nucleotide sequence coding for TBP-II or for a protein substantially homologous therewith. These DNA molecules may be genomic DNA, cDNA, synthetic DNA and combinations thereof.

See also claim 11 as originally filed,[1] which states:

> 11. A DNA molecule comprising the nucleotide sequence coding for the TNF Binding Protein TBP-II of claim 1 or to a protein homolog therewith.

Finally, reference is made to page 10, lines 13-19, of the present specification which states:

> Once one or more suitable peptide fragments have been sequenced or a partial sequence of the protein is determined, the DNA sequences capable of encoding them are examined. Due to the degeneration of the genetic code, more than one codon may be used to encode a particular amino acid and one or more different oligonucleotides can be produced, each of which would be capable of encoding the TBP-II peptide fragments (Watson, J.D., in: Molecular Biology of the Gene, 3rd ed., W.A. Benjamin, Inc. Menlo Park, CA (1977), pp,. 356-357).

[1] Original claims constitute their own description; *In re Koller*, 204 USPQ 702 (CCPA 1980).

The examiner has not disputed appellant's statement that there is adequate written description in the present specification to show that applicants were in possession of TBP-II. Furthermore, the examiner has conceded that the amino acid sequence of TBP-II is an inherent property of adequately described TBP-II (see the Examiner's Answer at page 8, lines 10-11, page 9, line 17, and page 12, line 7). Thus, for the purpose of the written description requirement, regardless of whether or not the amino acid sequence of TBP-II was set forth in full in the specification, it is apparent that applicants were in possession of that inherent amino acid sequence at the time the application was filed (see MPEP §2163.07(a)).

The above-quoted portions of the specification clearly state that applicants considered the DNA which encodes the TBP-II amino acid sequence to be part of their invention, including not only the genomic DNA and cDNA, but also synthetic DNA, i.e., any DNA sequence which encodes TBP-II. Further, the present specification incorporates by reference the genetic code by reference to the Watson publication (a copy of the cited pages is attached hereto as Exhibit A), which sets forth such genetic code in Table 13-7 at page 356, which page was specifically referred to at page 10, line 19.

While the disclosure at page 10, lines 13-19, was specifically with respect to peptide fragments or a partial sequence of the protein, this disclosure, in conjunction with the disclosure at page 8 of synthetic DNA and in claim 11 as originally filed of any DNA which encodes TBP-II, is sufficient

disclosure to establish that applicants were in possession of all of those DNA sequences that encode the inherent full amino acid sequence. In other words, those of ordinary skill in the art reading the specification as a whole, and particularly the above-quoted sections, would understand that applicants were in possession of the claimed DNA.

As applicants stated in the application as filed that they considered such DNA sequence to be part of their invention, and as applicants taught how to deduce such sequence from the genetic code, and as the full amino acid sequence (from which the genus of encoding DNA sequences may be deduced) was inherently a part of the present specification, it is as if the nucleotide sequence were fully set forth. Thus, the specification establishes that applicants were in possession of such sequences, thereby satisfying the written description requirement of the first paragraph of 35 U.S.C. §112.

Certainly, at least for the ten amino acid partial sequence of TBP-II set forth in the present claims and in the specification as filed, the present specification effectively discloses all of the DNA sequences that encode that partial sequence due to the degeneration of the genetic code (which is set forth in the Watson reference cited at page 10 of the specification). The examiner states that no DNA sequence whatsoever is set forth in the present specification (see page 4, lines 5-6, of the Examiner's Answer, as well as page 8,

lines 1 and 2, and page 9, lines 15-16)[2]. At page 10 of the Examiner's Answer, lines 3-5, the examiner stated:

> In addition, the step of converting the amino acid data into nucleotides encoding said molecule was not performed and therefore appellant was not in possession of the claimed nucleic acids.

However, the present specification at page 10, lines 13-19, at least effectively discloses all of the nucleic acid sequences according to the genetic code referenced in Watson, each of which encode the partial sequence of the protein as is set forth elsewhere in the specification. At page 5 of the Examiner's Answer, lines 1-4, the examiner states:

> The Federal Circuit has held that if an inventor is "unable to envision the detailed constitution of a gene so as to distinguish it from other materials ... conception has not been achieved until reduction to practice has occurred", Amgen, Inc. v. Chugai Pharmaceutical Co, Ltd., 18 U.S.P.Q.2d 1016 (Fed. Cir. 1991).

Here, however, it is apparent from the disclosure at page 10, lines 13 to 19, in conjunction with the disclosure of a partial sequence of the protein and the reference to the pages of Watson that contain the genetic code, that the present applicants were able to envision the detailed constitution of at least a portion of the DNA sequence encoding TBP-II so as to distinguish it from other materials. Thus, in this case, conception has been achieved and possession demonstrated. This implicit disclosure in the specification of all of the DNA

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[2] The examiner also states in this context that the whole protein has more than 250 amino acids or is encoded by 750 nucleic acids. This is incorrect. In fact, urinary TBP-II is substantially smaller, i.e., 185-192 amino acids (see, for example, Fig. 5 of U.S. patent 6,232,446 which is attached hereto as Exhibit B).

sequences that encode the disclosed ten amino acid partial sequence of TBP-II, combined with the disclosure at page 8 and claim 11 as originally filed that the invention comprehends any DNA that encodes TBP-II and the admitted fact that the full amino acid sequence is an inherent property of the TBP-II that was sufficiently identified by other properties (including partial amino acid sequence) in the present specification, establishes that the present specification is sufficient to comply with the written description requirement of the first paragraph of 35 U.S.C. §112 for the present claims.

This is clearly not an issue of a non-disclosed but obvious invention. Here, the above-quoted portions of the specification establish that applicants clearly disclosed that the claimed DNA was part of their invention. Furthermore, a set of partial DNA sequences are effectively disclosed at page 10, lines 13-19. This was sufficient to enable one to envision the detailed constitution of the genus of DNA sequences sufficiently to distinguish them from other sequences. Thus, the cases cited by the examiner about obviousness being insufficient for written description are inapposite.

The "Revised Interim Written Description Guidelines Training Materials" published by the PTO, and which are believed to still be applicable, have been studied to find how the PTO instructs its examiners to handle written description issues. While there do not appear to be any examples directly on point, the one which is most analogous to the present situation deals with an unexemplified antibody, discussed in

Example 16 of the Training Materials (copy of preamble and Example 16 being attached hereto as Exhibit C). The fact situation in that training example is that the specification teaches that antigen X has been isolated and is useful for detection of HIV infections. The specification teaches purified antigen X and provides characterization of the antigen by molecular weight. The specification also provides a clear protocol by which antigen X was isolated. As here with respect to the DNA molecule, the specification in the training example contemplated but did not teach in an example antibodies which specifically bind to antigen X and asserted that these antibodies can be used in immunoassays to detect HIV. The claim in question reads:

> **Claim:** An isolated antibody capable of binding to antigen X.

The "Analysis" and "Conclusions" sections of this training example read:

> **Analysis:**
>
> A review of the full content of the specification indicates that antibodies which bind to antigen X are essential to the operation of the claimed invention. The level of skill and knowledge in the art of antibodies at the time of filing was such that production of antibodies against a well-characterized antigen was conventional. This is a mature technology where the level of skill is high and advanced.
>
> The claim is directed to any antibody which is capable of binding to antigen X.
>
> A search of the prior art indicates that antigen X is novel and unobvious.
>
> Considering the routine art-recognized method of making antibodies to fully

> characterized antigens, the well defined structural characteristics for the five classes of antibody, the functional characteristics of antibody binding, and the fact that the antibody technology is well developed and mature, one of skill in the art would have recognized that the spectrum of antibodies which bind to antigen X were implicitly disclosed as a result of the isolation of antigen X.
>
> **Conclusion:** The disclosure meets the requirement under 35 USC 112 first paragraph as providing an adequate written description of the claimed invention.

Here, it is routine and art-recognized to identify the set of DNA sequences which correspond to a fully characterized protein whose amino acid sequence is inherent. Thus, one of skill in the art would have recognized that the spectrum of DNA sequences which code for the amino acid sequence of TBP-II was implicitly disclosed as a result of the isolation of TBP-II, just as was the spectrum of antibodies which bind to antigen X as a result of the isolation of antigen X in the above training example.

It is understood that training examples are not binding on the Board, however, they shed light on how the PTO instructs examiners to construe the written description requirement and the logic thereof is consistent with that argued herein. It is consistent with the case law and should be followed herein.

Accordingly, for these, as well as the reasons set forth in appellants' main Brief on Appeal, reversal of the examiner and withdrawal of the rejection of claims 11-13, 35-38, 43, 44, 46-49, 51-54, 56-61, 63 and 64 under the first paragraph of 35 U.S.C. §112 are respectfully urged.

The "Polypeptides" Described at Page 7, Lines 18-22, are the "Proteins Substantially Homologous Therewith" of Claim 11 as Originally Filed

The present specification contains an adequate written description of the claimed DNA molecules encoding a fragment of TBP-II that has the ability to inhibit the cytotoxic effect of TNF. The present specification at page 4, lines 15-17, states:

> Another object of the invention is the production of TBP-II by recombinant DNA techniques, including the preparation of DNA sequences coding for TBP-II or for a protein substantially homologous therewith, ... [emphasis added]

In addition, claim 11 as originally filed reads:

> 11. A DNA molecule comprising the nucleotide sequence coding for the TNF Binding Protein TBP-II of claim 1 or to a protein homolog therewith. [emphasis added]

Appellant has argued that the term "protein substantially homologous therewith" or "a protein homolog therewith" must be interpreted in accordance with the disclosure of page 7, lines 18-22, of the specification which states:

> The present invention encompasses a protein comprising the above sequence, herein referred to as TBP-II, as well as any other polypeptide in which one or more amino acids in the structure of natural TBP-II are deleted or replaced with other amino acids, or one or more amino acids are added thereto, as long as they have human TBP-II activity.

In response to this, the examiner takes the position that, because the last quoted passage refers to the modified sequences which maintain human TBP-II activity as "any other polypeptide", those of ordinary skill in the art would not understand that this is an appropriate definition of the

previously-used term "proteins substantially homologous therewith". The examiner states that this passage "clearly differentiates between protein (intact molecule) and polypeptide (shorter than intact molecule)". However, this statement is not correct. The term "any other polypeptide" as used in the above-quoted sentence refers not only to shorter sequences, but also longer sequences having added residues and sequences of the same length in which one or more amino acids have been substituted for the original residues. Thus, there is clearly no length distinction in the terms "protein" and "polypeptide" as used in this sentence.

Furthermore, by the term "any other polypeptide" it is clear that the TBP-II protein is also considered to be a polypeptide. Indeed, all proteins are polypeptides. It is believed that the examiner is making a hypertechnical distinction which would not be recognized by one of ordinary skill in the art. One of ordinary skill in the art looking for a reasonable meaning for the term "protein substantially homologous therewith" would readily consider that the modified sequences in the above-quoted passage, which modified sequences maintain human TBP-II activity, are the "proteins substantially homologous therewith" disclosed at page 4, or the "protein homolog therewith" as set forth in claim 11 as originally filed.

Accordingly, a reasonable interpretation of the specification will show that the present inventors were, indeed, in possession of the invention directed to DNA encoding

active fragments of TBP-II at the time that the application was filed. Reversal of the examiner and withdrawal of this rejection are also respectfully urged.

Withdrawal of the Rejection of Claim 63

It is noted with appreciation that the examiner has withdrawn the separate rejection of claim 63 under 35 U.S.C. §112, first paragraph, leaving only the first two issues set forth on page 17 of appellant's main brief for reconsideration by this Board.

CONCLUSION

Accordingly, for the reasons provided in appellant's main Brief on Appeal as supplemented by the reasons presented herein, reversal of the examiner and allowance of all of the claims now present in the case are earnestly solicited.

Respectfully submitted,

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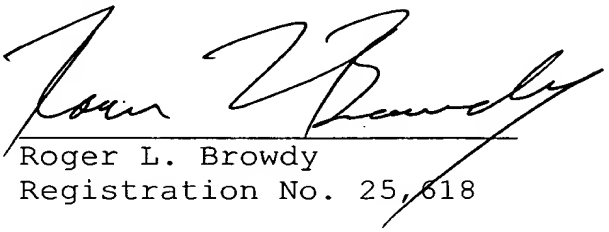
Attorneys for Appellant(s)

By 
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Molecular Biology of the Gene 3RD EDITION

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With illustrations by
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EXHIBIT A

Table 13–6 Assignment of Codon Orders Using Regular Copolymers Building from Four Bases

Copolymer	Amino Acids Incorporated	Codon Assignments
UAU|CUA|UCU|AUC|UAU . . .	Tyrosine	5′UAU3′
	Leucine	CUA
	Serine	UCU
	Isoleucine	AUC
UUA|CUU|ACU|UAC|UUA . . .	Leucine	UUA
	Leucine	CUU
	Threonine	ACU
	Tyrosine	UAC

have been analyzed, giving the results shown in Table 13–5. Only a few polymers having repeating tetranucleotide sequences have been looked at so far. The codon assignments obtained from two of them are revealed in Table 13–6. The sum of all these observations permits the definite assignments of specific amino acids to 61 out of the possible 64 codons (Table 13–7). The remaining three codons, as shown in Table 13–7, code for chain termination.

Table 13–7 The Genetic Code

First Position (5′ End)	Second Position U	C	A	G	Third Position (3′ End)
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Term[a]	Term	A
	Leu	Ser	Term	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	GluN	Arg	A
	Leu	Pro	GluN	Arg	G
A	Ileu	Thr	AspN	Ser	U
	Ileu	Thr	AspN	Ser	C
	Ileu	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

[a] Chain terminating (formerly called "nonsense").

Jsing Regular

ds ed	Codon Assignments
	5'UAU3'
	CUA
	UCU
	AUC
	UUA
	CUU
	ACU
	UAC

s shown in Table
eating tetranucleo-
far. The codon as-
n are revealed in
'ations permits the
acids to 61 out of
ıe remaining three
for chain termina-

	Third Position (3' End)
›ys	U
›ys	C
'erm	A
'rp	G
\rg	U
\rg	C
\rg	A
\rg	G
›er	U
›er	C
\rg	A
\rg	G
›ly	U
›ly	C
›ly	A
›ly	G

THE CODE IS DEGENERATE

Many amino acids are selected by more than one codon (degeneracy). For example, both (UUU) and (UUC) code for phenylalanine, while serine is coded by (UCU), (UCC), (UCA), (UCG), (AGU), and (AGC). The present data suggest that when the first two nucleotides are identical, the third nucleotide can be either cytosine or uracil and the codon will still code for the same amino acid. Often adenine and guanine are similarly interchangeable. However, not all degeneracy seems to be based on equivalence of the first two nucleotides. Leucine, for example, seems to be coded by (UUA) and (UUG), as well as by (CUU), (CUC), (CUA), and (CUG) (Figure 13–4).

Codon degeneracy, especially the frequent third-place equivalence of cytosine and uracil or guanine and adenine, underlies the fact that the AT/GC ratios can show very great variations (see Chapter 9) without correspondingly large changes in the relative proportion of amino acids found in these organisms. The original explanation was that these similarities in amino acid composition were meaningless, reflecting the sequences of only those genes coding for proteins present in large quantities. But as the analysis of more individual proteins revealed no real correlation between amino acid composition and evolutionary position, this interpretation became untenable.

It was also at first guessed that a specific anticodon would exist for every codon. If so, at least 61 different tRNA's, possibly with an additional three for the chain-terminating codons, would be present. Evidence soon began to appear, however, that highly purified tRNA species of known sequence (e.g., alanyl-tRNA) could recognize several different codons. Several cases also arose where an anticodon base was not one of the four regular ones, but a fifth base, inosine. Like all the other minor tRNA bases, this arises through enzymatic modification of a base present in an otherwise completed tRNA. The base from which it is derived is adenine, whose 6-carbon is deaminated to give the 6-keto group of inosine.

THE WOBBLE IN THE ANTICODON

To explain these observations, the wobble concept was devised. It states that the base at the 5′ end of the anticodon is not as spatially confined as the other two, allowing it to form hydrogen bonds with any of several bases located at the 3′ end of a codon. Not all combinations are possible, with pairing restricted to those shown

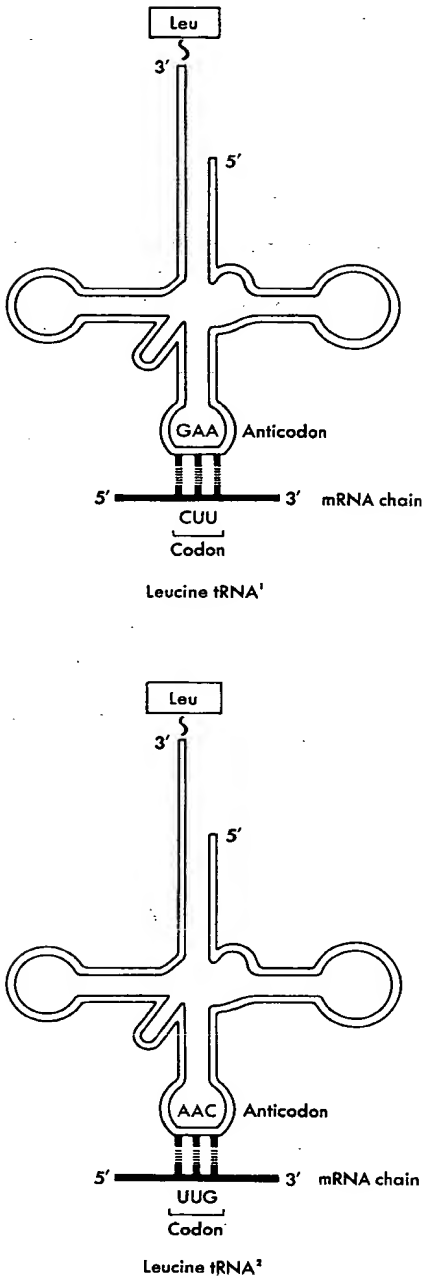

Figure 13–4
Two different tRNA molecules which accept leucine residues. Each recognizes a different code word.

US006232446B1

(12) **United States Patent**
Wallach et al.

(10) **Patent No.: US 6,232,446 B1**
(45) **Date of Patent: *May 15, 2001**

(54) **TNF LIGANDS**

(75) Inventors: **David Wallach**, Rehovot (IL); **Jacek Bigda**, Gdansk (PL); **Igor Beletsky**, Pushino (RU); **Igor Mett**, Rehovot (IL); **Hartmut Engelmann**, Munich (DE)

(73) Assignee: **Yeda Research and Development Co. Ltd.**, Rehovot (IL)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **08/477,347**

(22) Filed: **Jun. 7, 1995**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/450,972, filed on May 25, 1995, now abandoned, and a continuation-in-part of application No. 07/930,443, filed on Aug. 19, 1992, said application No. 07/930,443, is a continuation of application No. 07/524,263, filed on May 16, 1990, now abandoned, said application No. 08/450,972, is a continuation of application No. 08/115,685, filed on Sep. 3, 1993, now abandoned.

(30) **Foreign Application Priority Data**

May 18, 1989	(IL)	90339
Aug. 6, 1989	(IL)	91229
Apr. 6, 1990	(IL)	94039
Sep. 3, 1992	(IL)	103051
Jul. 8, 1993	(IL)	106271

(51) **Int. Cl.**[7] **C07K 16/28**; C12N 5/12

(52) **U.S. Cl.** **530/388.22**; 530/387.1; 530/387.9; 530/388.1; 530/388.2; 530/389.1; 435/326; 435/331; 435/332; 435/334; 435/346

(58) **Field of Search** 424/130.1, 131.1, 424/133.1, 134.1, 135.1, 139.1, 141.1, 145.1, 144.1, 152.1, 153.1, 154.1; 514/218, 12; 530/387.1–387.3, 387.9, 388.1, 388.22, 388.23, 388.7, 388.73, 388.75, 388.2; 435/326, 332, 333, 331, 335

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(List continued on next page.)

Primary Examiner—Phillip Gambel
(74) *Attorney, Agent, or Firm*—Browdy and Neimark

(57) **ABSTRACT**

Antibodies to tumor necrosis factor receptors (TNF-Rs) are disclosed together with methods of producing them. The antibodies are preferably those which inhibit the cytotoxic effect of TNF but not its binding to the TNF-Rs. Most preferably, the antibodies bind to an extracellular domain of the C-terminal cysteine loop of the p75 TNF receptor, which loop consists of the amino acid sequence Cys-185 to Thr-201 of SEQ ID NO:3.

15 Claims, 17 Drawing Sheets

EXHIBIT B

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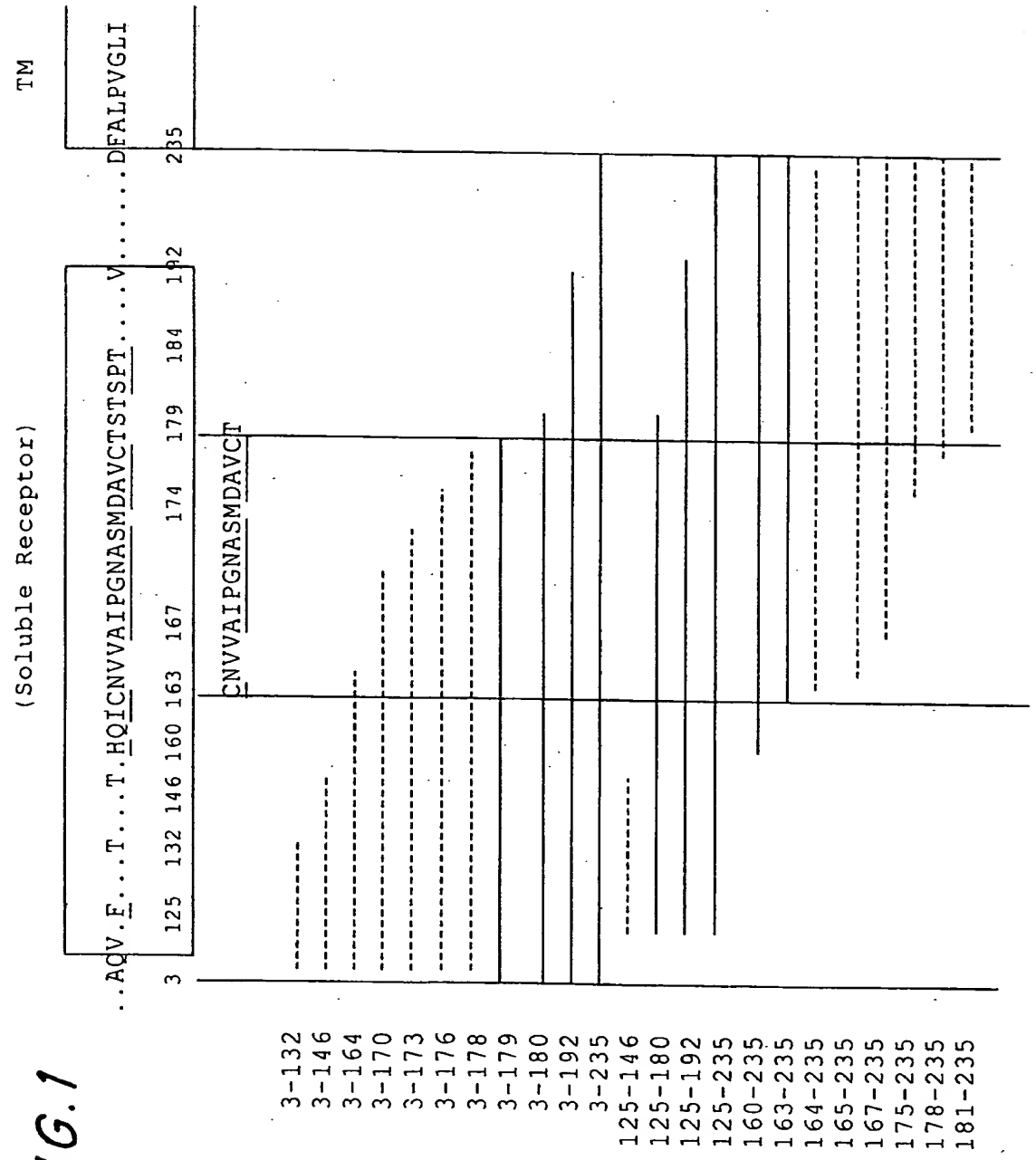
(Soluble Receptor)
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FIG. 1

FIG. 2

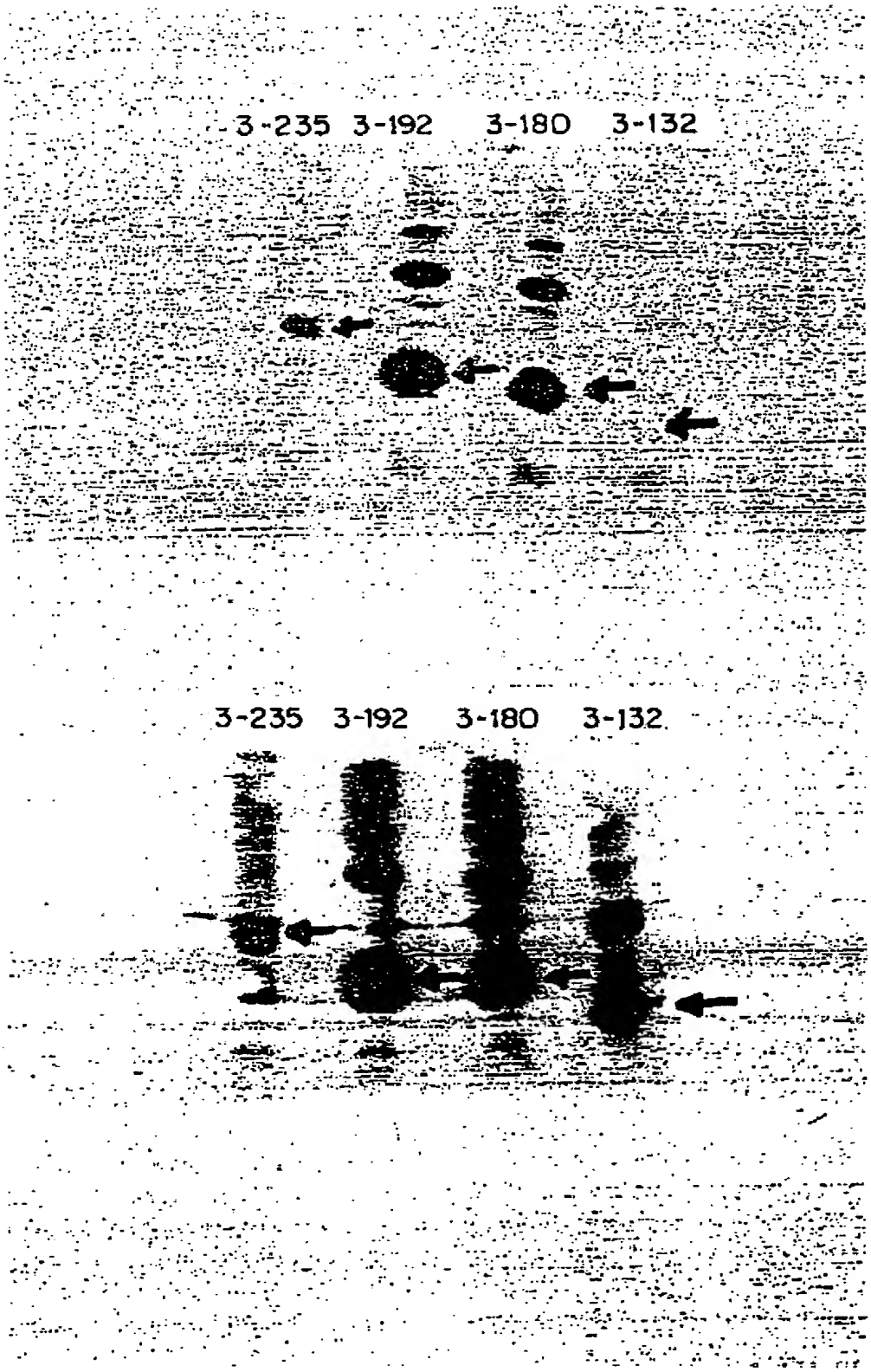
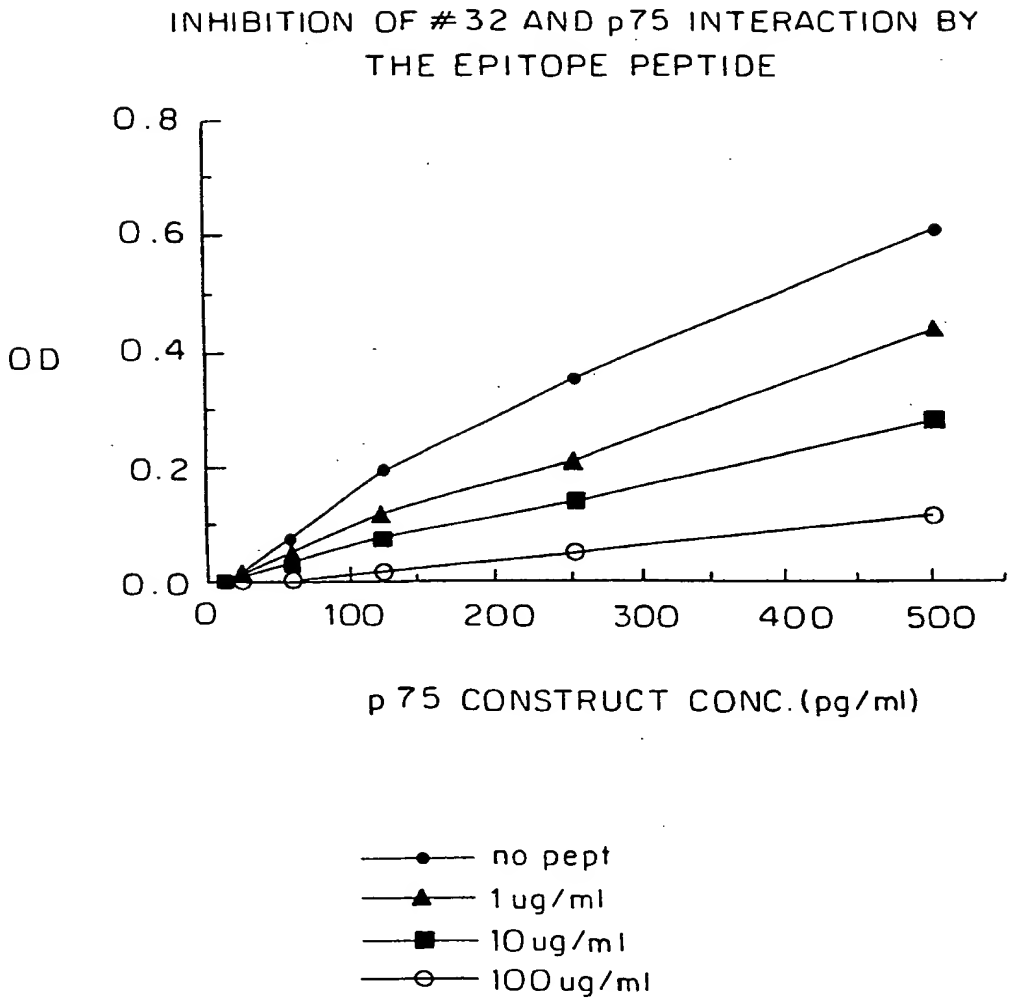


FIG. 3


INHIBITION OF #32 AND p75 INTERACTION BY THE EPITOPE PEPTIDE
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0.6
0.4
0.2
0.0
0
100
200
300
400
500
p75 CONSTRUCT CONC.(pg/ml)
no pept
1 ug/ml
10 ug/ml
100 ug/ml

FIG. 4

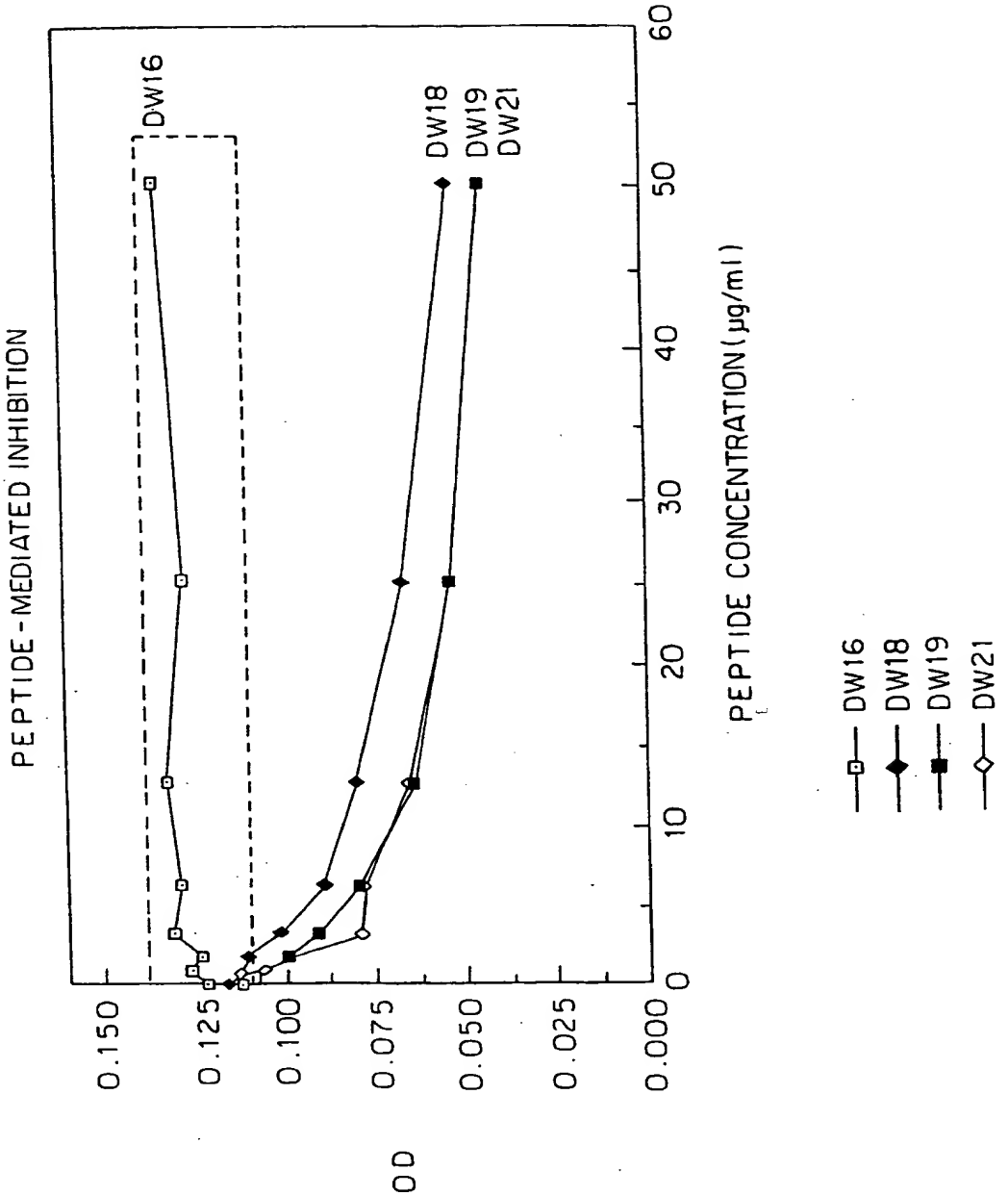

PEPTIDE-MEDIATED INHIBITION
OD
0.150
0.125
0.100
0.075
0.050
0.025
0.000
0
10
20
30
40
50
60
PEPTIDE CONCENTRATION(μg/ml)
DW16
DW18
DW19
DW21

FIG. 5A

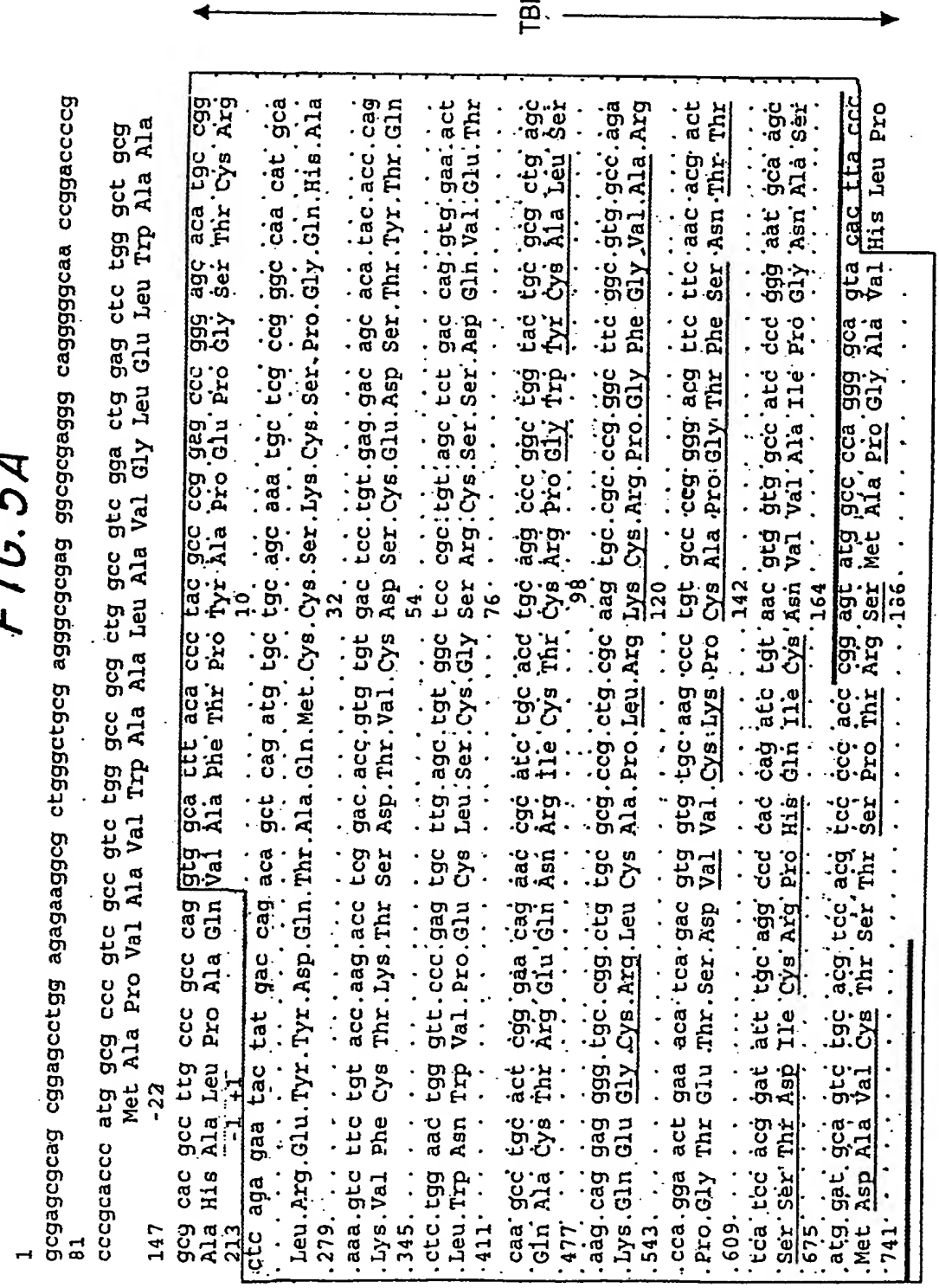


FIG.5B

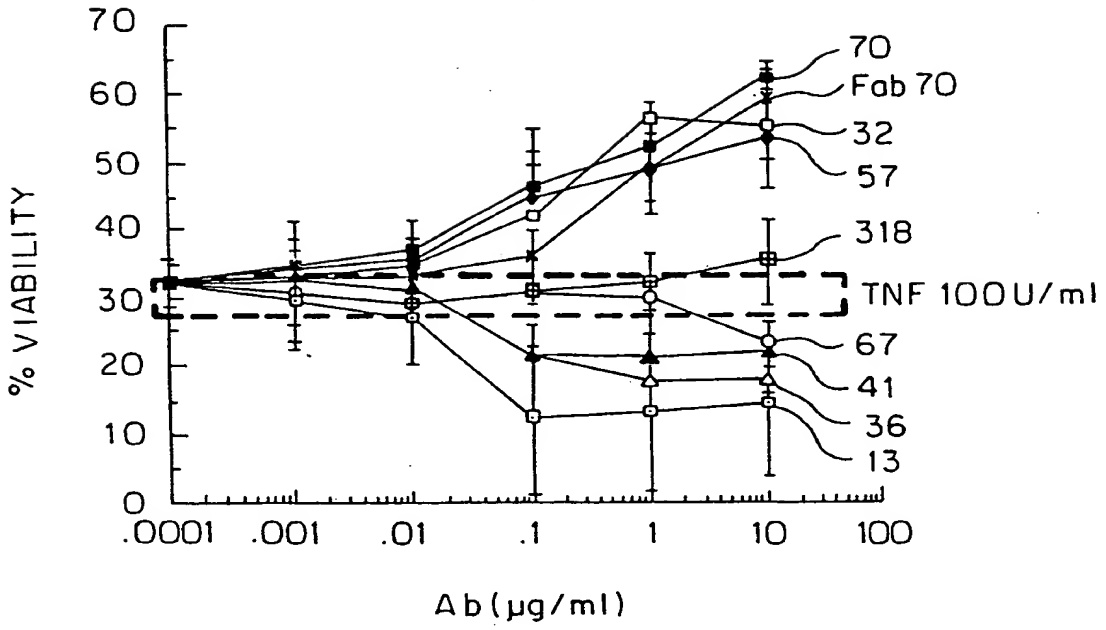
```
cag cca gtg tcc aca cga tcc caa cac acg cag cca act cca gaa ccc agc act gct cca agc acc
Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr
807                                                 208
tcc ttc ctg ctc cca atg ggc ccc agc ccc cca gct gaa ggg agc act ggc gac ttc gct ctt cca
Ser Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp Phe Ala Leu Pro
873                                                 230
gtt gga ctg att gtg ggt gtg aca gcc ttg ggt cta cta ata ata gga gtg gtg aac tgt gtc atc
Val Gly Leu Ile Val Gly Val Thr Ala Leu Gly Leu Leu Ile Ile Gly Val Val Asn Cys Val Ile
939                                                 252
atg acc cag gtg aaa aag aag ccc ttg tgc ctg cag aga gaa gcc aag gtg cct cac ttg cct gcc
Met Thr Gln Val Lys Lys Lys Pro Leu Cys Leu Gln Arg Glu Ala Lys Val Pro His Leu Pro Ala
1005                                                274
gat aag gcc cgg ggt aca cag ggc ccc gag cag cag cac ctg ctg atc aca gcg ccg agc tcc agc
Asp Lys Ala Arg Gly Thr Gln Gly Pro Glu Gln Gln His Leu Leu Il- Thr Ala Pro Ser Ser Ser
1071                                                296
agc agc tcc ctg gag agc tcg gcc agt gcg ttg gac aga agg gcg ccc act cgg aac cag cca cag
Ser Ser Ser Leu Glu Ser Ser Ala Ser Ala Leu Asp Arg Arg Ala Pro Thr Arg Asn Gln Pro Gln
1137                                                318
gca cca ggc gtg gag gcc agt ggg gcc gcg gag gcc cgg gcc agc acc ggg agc tca gat tct tcc
Gly Ala Gly Glu Ala Arg Ala Ser Thr Gly Ser Ser Asp Ser Ser Pro Gly Gly His Gly Thr Gln
1203                                                340
ctt ggt ggc cat ggg acc cag gtc aat gtc acc tgc atc gtg aac gtc tgt agc agc tct gac cac
Ala Pro Gly Val Glu Ala Ser Val Asn Val Thr Cys Ile Val Asn Val Cys Ser Ser Ser Asp His
1269                                                362
agc tca cag tgc tcc tcc caa gcc agc tcc aca atg gga gac aca gat tcc agc ccc tcg gag tcc
Ser Ser Gln Cys Ser Ser Gln Ala Ser Ser Thr Met Gly Asp Thr Asp Ser Ser Pro Ser Glu Ser
1335                                                384
ccg aag gac gag cag gtc ccc ttc tcc aag gag gaa tgt gcc ttt cgg tca cag ctg gag acg cca
Pro Lys Asp Glu Gln Val Pro Phe Ser Lys Glu Glu Cys Ala Phe Arg Ser Gln Leu Glu Thr Pro
1401                                                406
gag acc ctg ctg ggg agc acc gaa gag aag ccc ctg ccc ctt gga gtg cct gat gct ggg atg aag
Glu Thr Leu Leu Gly Ser Thr Glu Glu Lys Pro Leu Pro Leu Gly Val Pro Asp Ala Gly Met Lys
1467                                                428
ccc agt taa ccaggccggt gtgggctgtg tcgtagccaa ggtgggctga gccctggcag gatgaccctg cgaaggggc
Pro Ser End
    439
```

TRANSMEMBRANE DOMAIN

FIG. 5C

```
1545
cctggtcctt ccaggccccc accactagga ctctgaggct ctttctgggc caagttcctc tagtgccctc cacagccgca
gcctccctct gacctgcagg ccaagagcag aggcagcgag ttggggaaag cctctgctgc catggtgtgt ccctctcgga
aggctggctg ggcatggacg ttcggggcat gctggggcaa gtccctgact ctctgtgacc tgccccgccc agctgcacct
gccagcctgg cttctgggtt ttgttttgtt tgtttgtttg ttgtttttgtt tctccccctg ggctctgccc
agctctggct tccagaaaac cccagcatcc ttttctgcag aggggctttc tggagaggag ggatgctgcc tgagtcaccc
atgaagacag gacagtgctt ctgcctgagg cagagactgc gggatgggtcc tggggctctg tgtagggagg aggtggcagc
cctgtagga acggggtcct tcaagttagc tcaggaggct tggaaagcat cacctcaggc caggtgcagt ggctcacgcc
tatgatccca gcactttggg aggctgaggc gggtggatca cctgagggtta ggagttcgag accagcctgg ccaacatggt
aaaaccccat ctctactaaa aatacagaaa ttagccgggc .....3683
                                                    acctcaggc caggtgcagt ggctcacgcc
                                                    2075
```

FIG. 6


70
60
50
40
30
20
10
0
% VIABILITY
.0001
.001
.01
.1
1
10
100
Ab (µg/ml)
70
Fab 70
32
57
318
TNF 100U/ml
67
41
36
13

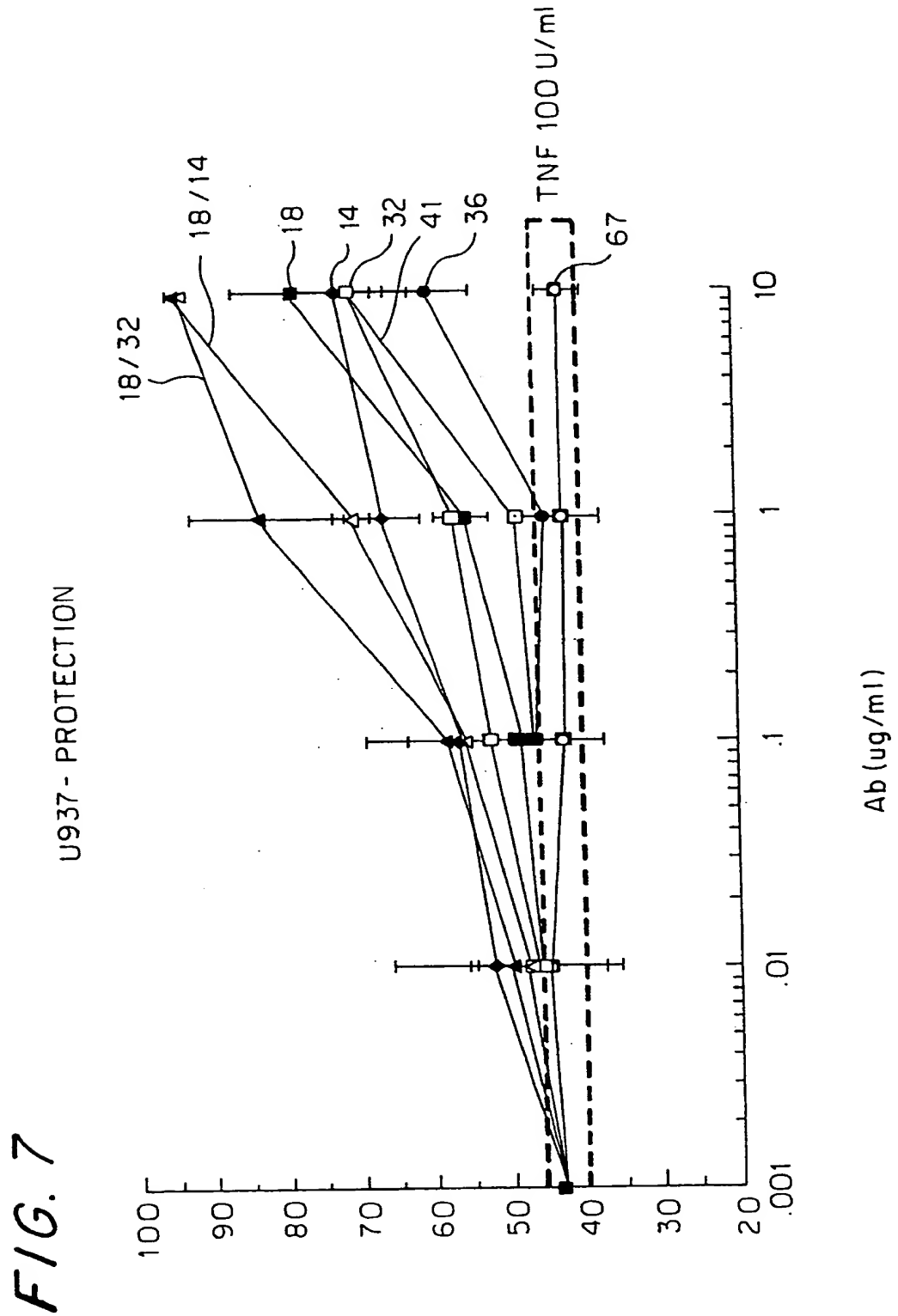

U937- PROTECTION
TNF 100 U/ml
18/14
18/32
18
14
32
41
36
67
100
90
80
70
60
50
40
30
20
.001
.01
.1
1
10
Ab (ug/ml)

FIG. 7

FIG. 8A

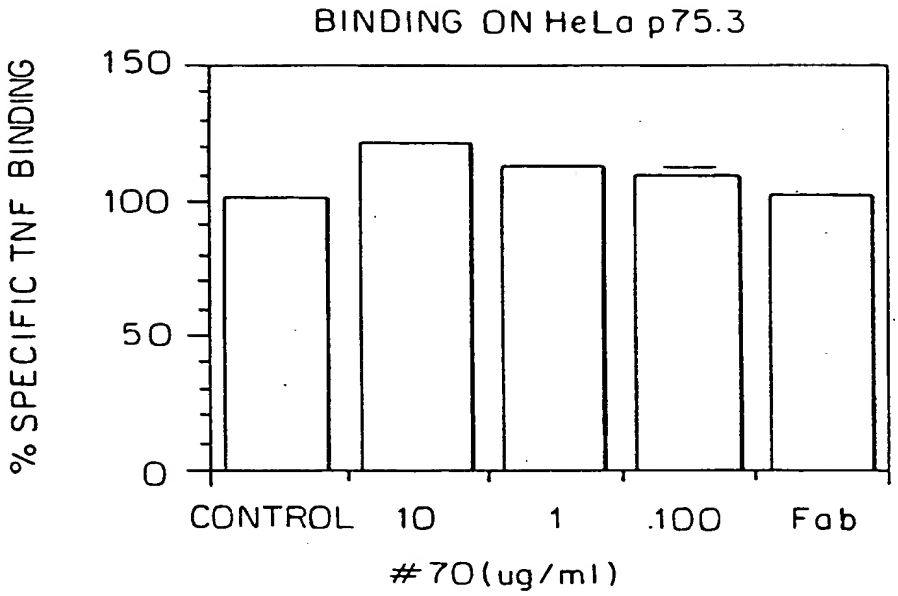

BINDING ON HeLa p75.3
% SPECIFIC TNF BINDING
150
100
50
0
CONTROL
10
1
.100
Fab
#70(ug/ml)

FIG. 8B

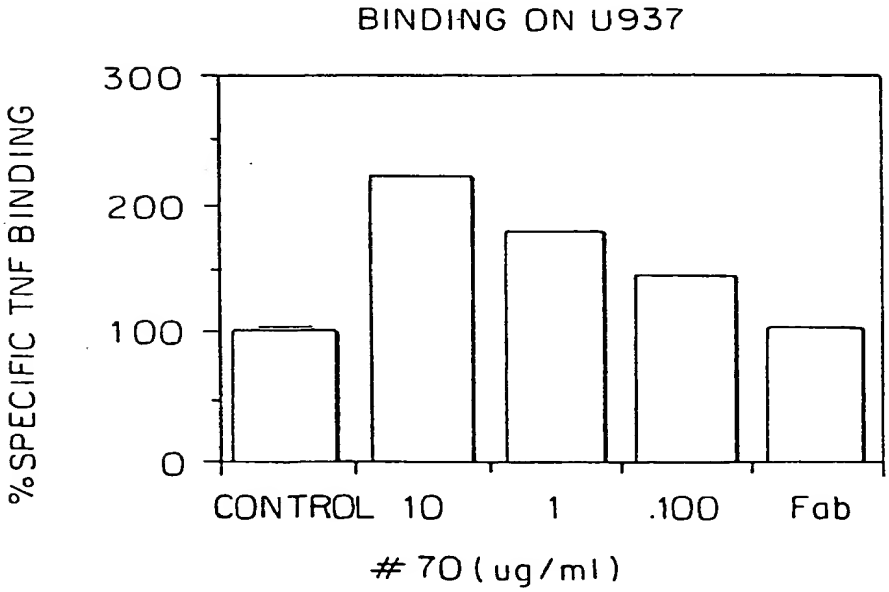

BINDING ON U937
% SPECIFIC TNF BINDING
300
200
100
0
CONTROL
10
1
.100
Fab
#70 (ug/ml)

FIG. 9A

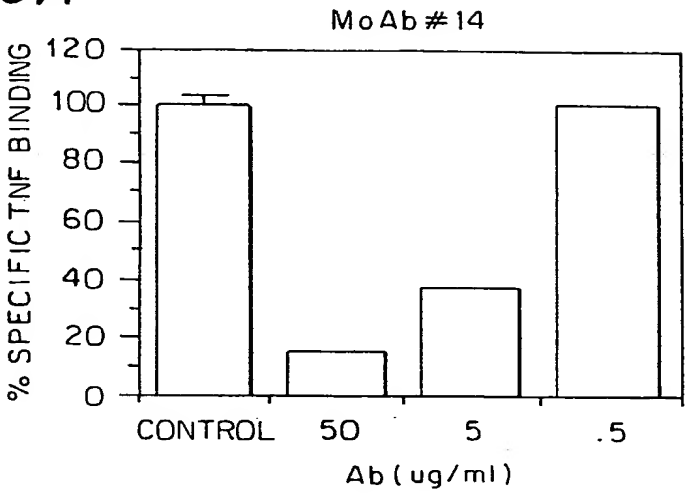


FIG. 9B

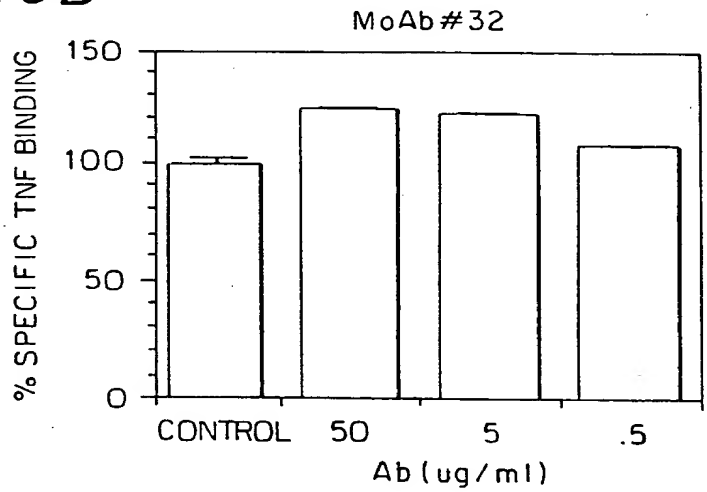


FIG. 9C

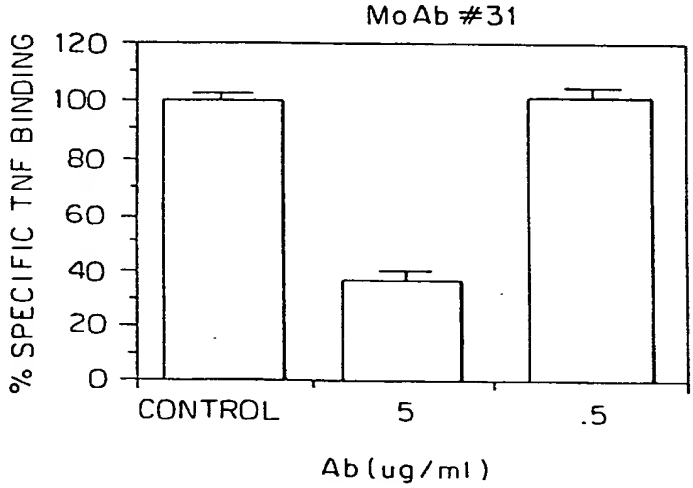


FIG. 9D

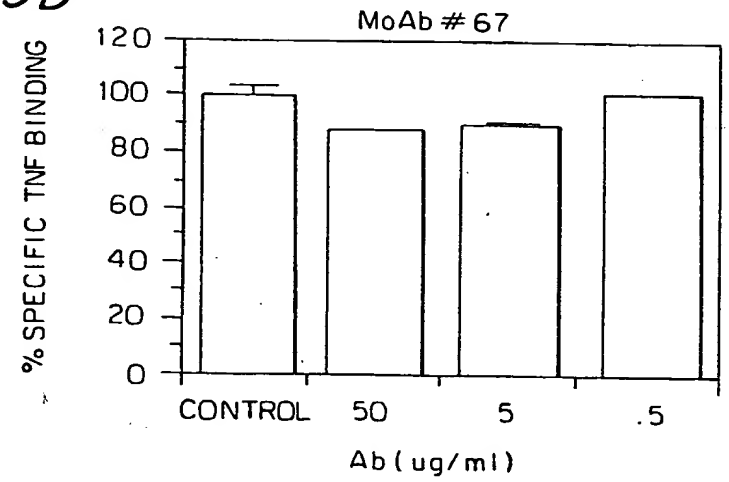


FIG. 9E

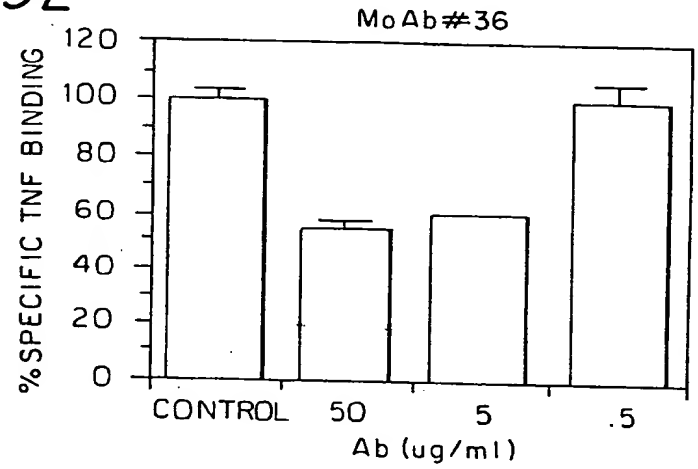


FIG. 9F

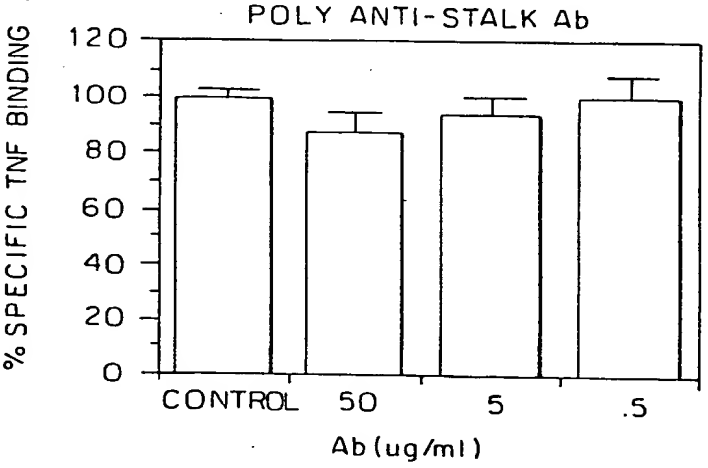


FIG. 10

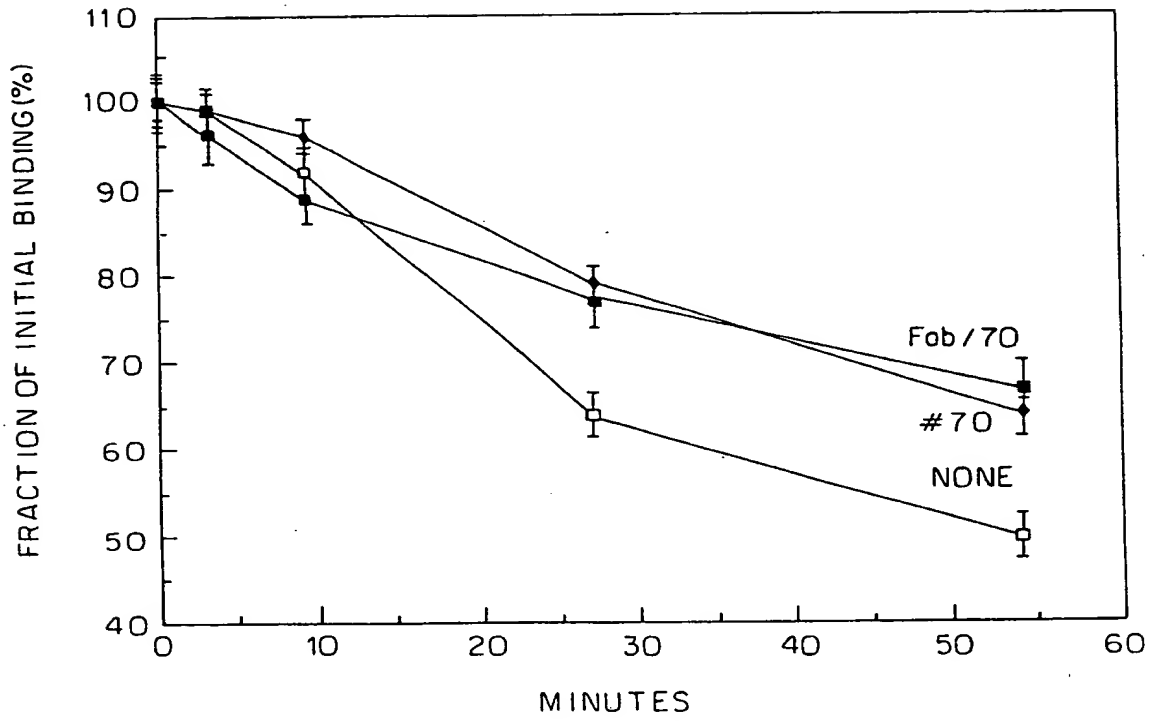

110
100
90
80
70
60
50
40
FRACTION OF INITIAL BINDING (%)
0
10
20
30
40
50
60
MINUTES
Fab / 70
#70
NONE

FIG. 11A

```
     1/1                                         31/11
#70  GTG AAA CTG CAG GAG TCT GGA CCT GAG CTG GTG AAG CCT GGG GCC TCA GTG AAG ATT TCC
     V   K   L   Q   E   S   G   P   E   L   V   K   P   G   A   S   V   K   I   S
     1/1                                         31/11
#32                              CCT GAG CTG GTG GCT CCT GGG GCC TCA GTG AAG ATT TCC
                                 P   E   L   V   A   P   G   A   S   V   K   I   S
     1/1                                         31/11
#57  GTG TCC CTG CAG GAG TCT GGG GGA GGC TTA GTG CAG CCT GGA GGG TCC CGG AAA CTC TCC
     V   S   L   Q   E   S   G   G   G   L   V   Q   P   G   G   S   R   K   L   S
-----------------------------------------------------------------------------------
     61/21                                       91/31
#70  TGC AAA ACT TCT GGC TTC GCA TTC AGT CAT TCT TGG ATG AAC TGG GTG AGG CAG AGG CCT
     C   K   T   S   G   F   A   F   S   H   S   W   M   N   W   V   R   Q   R   P
     61/21                                       91/31
#32  TGC AAA GCT TCT GGC TAC GCA TTC AGT CAC TCT TGG ATG AAC TGG GTG AAG CAG AGG CCT
     C   K   A   S   G   Y   A   F   S   H   S   W   M   N   W   V   K   Q   R   P
     61/21                                       91/31
#57  TGT GCA GCT TCT GGA TTC ACT TTC AGT AGC TTT GGA ATG CAC TGG GTT CGT CAG GCT CCA
     C   A   A   S   G   F   T   F   S   S   F   G   M   H   W   V   R   Q   A   P
-----------------------------------------------------------------------------------
     121/41                                      151/51
#70  GGA CAG GGT CTT GAA TGG ATT GGA CGG ATT TAT CCT GGA GAT GGA AAT ACT GAT TAC CCT
     G   Q   G   L   E   W   I   G   R   I   Y   P   G   D   G   N   T   D   Y   N
     121/41                                      151/51
#32  GGA AAG GGT CTT GAG TGG ATT GGA CGG ATT CAT CCT GGA GAT GGA GAC ACT GAC TAC AAT
     G   K   G   L   E   W   I   G   R   I   H   P   G   D   G   D   T   D   Y   N
     121/41                                      151/51
#57  GAG AAG GGG CTG GAG TGG GTC GCA TAC ATT AGT AGT GGC AGT AGT ACC CTC CAC TAT GCA
     E   K   G   L   E   W   V   A   Y   I   S   S   G   S   S   T   L   H   Y   A
-----------------------------------------------------------------------------------
```

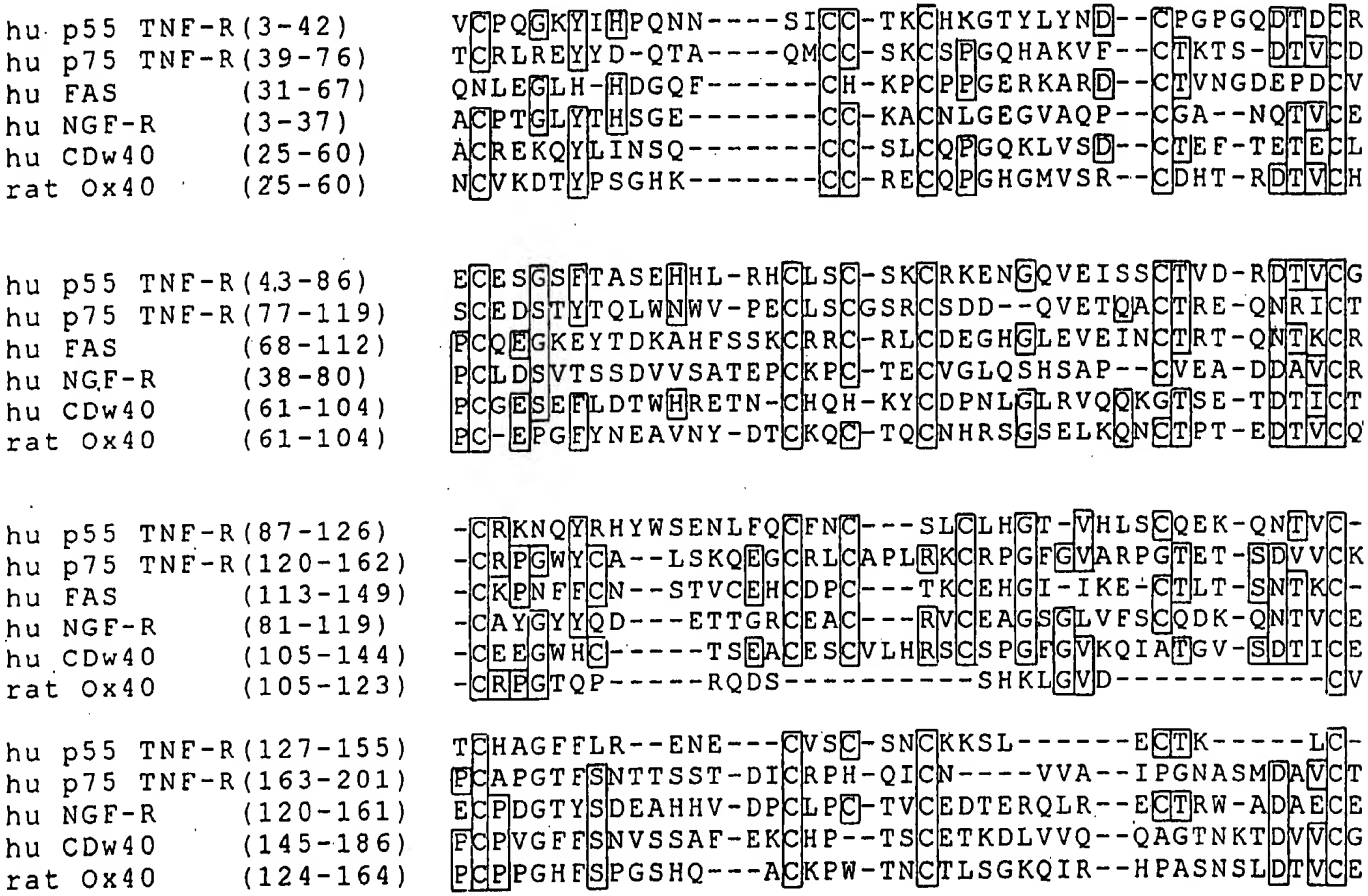
FIG. 11B

```
     181/61                                  211/71
#70  GGG AAG TTC CAG GGC CAG GCC ACA CTG ACT GCA GAC AAA TCT TCC AGC ACA GCC TAC ATG
     G   K   F   Q   G   Q   A   T   L   T   A   D   K   S   S   S   T   A   Y   M
     181/61                                  211/71
#32  GGG AAC TTC AGG GGC AAG GCC ACA CTG ACT GCA GAC ACA TCC TCC AGC TCA GCC TAC ATG
     G   N   F   R   G   K   A   T   L   T   A   D   T   S   S   S   S   A   Y   M
     181/61                                  211/71
#57  GAC ACA GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC AAT CCC AAG AAC ACG CTG TTC CTG
     D   T   V   K   G   R   F   T   I   S   R   D   N   P   K   N   T   L   F   L

     241/81                                  271/91
#70  CAA CTC TTC AGT CTG ACC TCT GTG GAC TCT GCG GTC TAT TTT TGT GCA CCC GGC CGT TGG
     Q   L   F   S   L   T   S   V   D   S   A   V   Y   F   C   A   P   G   R   W
     241/81
#32  CAG CTC AGC AGC CTG ACC TCT GTG GAT TCT GCG GTC TAC TTC TGT GCA CCC GGC CGT TGG
     Q   L   S   S   L   T   S   V   D   S   A   V   Y   F   C   A   P   G   R   W
     241/81                                  271/91
#57  CAA ATG AAA CTA CCC TCA CTA TGC TAT GGA CTA CTG GGG CCA AGG GAC CAC GGT CAC CGT
     Q   M   K   L   P   S   L   C   Y   G   L   L   G   P   R   D   H   G   H   R

     301/101                                 331/111
#70  TAC CTC GAA GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA
     Y   L   E   V   W   G   Q   G   T   T   V   T   V   S   S
     301/101
#32  TAC CTC GAG GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA
     Y   L   E   V   W   G   Q   G   T   T   V   T   V   S   S
     301/101
#57  CTC CTC Λ
     L   L
```

FIG. 12

```
                                                                  31/11
                                                TCC TCC CTG GCT ATG TCA GTA GGA CAG ATG GTC ACT
                                                S   S   L   A   M   S   V   G   Q   M   V   T
                                                                  91/31
61/21
ATG AGC TGC AAG TCC AGT CAG AGC CTT TTA ACT AGT AGC ACT CAA AAG AAC TCT TTG GCC
M   S   C   K   S   S   Q   S   L   L   T   S   S   T   Q   K   N   S   L   A
121/41                                                            151/51
TGG TAC CAG CAG ACA CCA GGA CAG TCT CCT AAA CTT CTG ATA TAC TTT GCA TCC ACT AGG
W   Y   Q   Q   T   P   G   Q   S   P   K   L   L   I   Y   F   A   S   T   R
181/61                                                            211/71
CTA TCT GGG GTC CCT GAT CGC TTC ATA GGC AGT GGA TCT GGG ACA GAT TTC ACT CTT ACC
L   S   G   V   P   D   R   F   I   G   S   G   S   G   T   D   F   T   L   T
241/81                                                            271/91
ATC AGC AGT GTG CAG GCT GAA GAC CTG GCA GAT TAC TTC TGT CAG CAA CAT TAT AGC ACT
I   S   S   V   Q   A   E   D   L   A   D   Y   F   C   Q   Q   H   Y   S   T
301/101                                                           331/111
CCA TTT ACG TTC GGC TCG GGG ACA AAG TTG GAA ATA GAG CGG GCT GAT GCT GCA CCA ACT
P   F   T   F   G   S   G   T   K   L   E   I   E   R   A   D   A   A   P   T
361/121
GTA TCC ATC TTC CCA CCA TCC A
V   S   I   F   P   P   S
```

FIG. 13


hu p55 TNF-R(3-42) VCPQGKYIHPQNN----SICC-TKCHKGTYLYND--CPGPGQDTDCR
hu p75 TNF-R(39-76) TCRLREYYD-QTA----QMCC-SKCSPGQHAKVF--CTKTS-DTVCD
hu FAS (31-67) QNLEGLH-HDGQF------CH-KPCPPGERKARD--CTVNGDEPDCV
hu NGF-R (3-37) ACPTGLYTHSGE-------CC-KACNLGEGVAQP--CGA--NQTVCE
hu CDw40 (25-60) ACREKQYLINSQ-------CC-SLCQPGQKLVSD--CTEF-TETECL
rat Ox40 (25-60) NCVKDTYPSGHK-------CC-RECQPGHGMVSR--CDHT-RDTVCH
hu p55 TNF-R(43-86) ECESGSFTASEHHL-RHCLSC-SKCRKENGQVEISSCTVD-RDTVCG
hu p75 TNF-R(77-119) SCEDSTYTQLWNWV-PECLSCGSRCSDD--QVETQACTRE-QNRICT
hu FAS (68-112) PCQEGKEYTDKAHFSSKCRRC-RLCDEGHGLEVEINCTRT-QNTKCR
hu NGF-R (38-80) PCLDSVTSSDVVSATEPCKPC-TECVGLQSHSAP--CVEA-DDAVCR
hu CDw40 (61-104) PCGESEFLDTWHRETN-CHQH-KYCDPNLGLRVQQKGTSE-TDTICT
rat Ox40 (61-104) PC-EPGFYNEAVNY-DTCKQC-TQCNHRSGSELKQNCTPT-EDTVCQ
hu p55 TNF-R(87-126) -CRKNQYRHYWSENLFQCFNC---SLCLHGT-VHLSCQEK-QNTVC-
hu p75 TNF-R(120-162) -CRPGWYCA--LSKQEGCRLCAPLRKCRPGFGVARPGTET-SDVVCK
hu FAS (113-149) -CKPNFFCN--STVCEHCDPC---TKCEHGI-IKE-CTLT-SNTKC-
hu NGF-R (81-119) -CAYGYYQD---ETTGRCEAC---RVCEAGSGLVFSCQDK-QNTVCE
hu CDw40 (105-144) -CEEGWHC-----TSEACESCVLHRSCSPGFGVKQIATGV-SDTICE
rat Ox40 (105-123) -CRPGTQP-----RQDS----------SHKLGVD----------CV
hu p55 TNF-R(127-155) TCHAGFFLR--ENE---CVSC-SNCKKSL------ECTK-----LC-
hu p75 TNF-R(163-201) PCAPGTFSNTTSST-DICRPH-QICN----VVA--IPGNASMDAVCT
hu NGF-R (120-161) ECPDGTYSDEAHHV-DPCLPC-TVCEDTERQLR--ECTRW-ADAECE
hu CDw40 (145-186) FCPVGFFSNVSSAF-EKCHP--TSCETKDLVVQ--QAGTNKTDVVCG
rat Ox40 (124-164) PCPPGHFSPGSHQ---ACKPW-TNCTLSGKQIR--HPASNSLDTVCE

TNF LIGANDS

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a continuation-in-part of U.S. application Ser. No. 07/930,443, filing date Aug. 19, 1992, and a continuation-in-part of U.S. application Ser. No. 08/450,972, filed May 25, 1995, now abandoned. The entire contents of both of said applications are hereby incorporated herein by reference. Application Ser. No. 07/930,443 is a continuation of application Ser. No. 07/524,263, filed May 16, 1990, now abandoned. Application Ser. No. 08/450,972, filed May 25, 1995, is a continuation of application Ser. No. 08/115,685, filed Sep. 3, 1993, now abandoned.

FIELD OF THE INVENTION

The present invention relates to ligands to Tumor Necrosis Factor receptors (TNF-Rs) which inhibit the effect of TNF but not its binding to the TNF-Rs, as well as to ligands interacting with other receptors of the TNF/NGF receptor family.

BACKGROUND OF THE INVENTION

Tumor necrosis factor (TNF) is a pleiotropic cytokine, produced by a number of cell types, mainly by activated macrophages. It is one of the principal mediators of the immune and inflammatory response. Interest in its function has greatly increased, recently, in view of evidence of the involvement of TNF in the pathogenesis of a wide range of disease states, including endotoxin shock, cerebral malaria and graft-versus-host reaction. Since many of the effects of TNF are deleterious to the organism, it is of great interest to find ways of blocking its action on host cells. An evident target for such intervention are the molecules to which TNF has to bind in order to exert its effects, namely the TNF-Rs. These molecules exist not only in cell-bound, but also in soluble forms, consisting of the cleaved extra-cellular domains of the intact receptors (see Nophar et al., EMBO Journal, 9(10):3269–78, 1990). The soluble receptors maintain the ability to bind TNF, and thus have the ability to block its function by competition with surface receptors.

Another method of TNF inhibition based on the principle of competing with cell-bound molecules, is the use of antibodies recognizing TNF receptors and blocking the ligand binding.

The cell surface TNF-Rs are expressed in almost all cells of the body. The various effects of TNF, the cytotoxic, growth-promoting and others, are all signalled by the TNF receptors upon the binding of TNF to them. Two forms of these receptors, which differ in molecular size: 55 and 75 kilodaltons, have been described, and well be called herein p55 and p75 TNF-R, respectively. It should be noted, however, that there exist publications which refer to these receptors also as p60 and p80.

The TNF-Rs belong to a family of receptors which are involved in other critical biological processes. Examples of these receptors are the low affinity NGF receptor, which plays an important role in the regulation of growth and differentiation of nerve cells. Several other receptors are involved in the regulation of lymphocyte growth, such as CDw40 and some others. Another member of the family is the FAS receptor also called APO, a receptor which is involved in signalling for apoptosis and which, based on a study with mice deficient in its function, seems to play an important role in the etiology of a lupus-like disease. Herein, this family of receptors is called "TNF/NGF receptor family".

One of the most striking features of TNF compared to other cytokines, thought to contribute to the pathogenesis of several diseases, is its ability to elicit cell death. The cell-killing activity of TNF is thought to be induced by the p55 receptor. However, this p55 receptor activity can be assisted by the p75 receptor, through a yet unknown mechanism.

Parent application number 07/524,263 and European patent publication nos. 0,398,327 and 0,412,486 disclose antibodies to the soluble TNF-Rs. These antibodies were found to recognize the soluble TNF-Rs and to inhibit the binding of TNF to the TNF-Rs on the cell surface. Monovalent F(ab) fragments blocked the effect of TNF, while intact antibodies were observed to mimic the cytotoxic effect of TNF.

SUMMARY OF THE INVENTION

The present invention provides a ligand to a member of the TNF/NGF receptor family, which binds to the region of the C-terminal cysteine loop of such a receptor.

Preferably this region includes the amino acid sequence cys-163 to thr-179 in the p75 TNF-R or a corresponding region in another member of the TNF/NGF family.

Preferably, the receptor is the TNF-R, in particular the p75 TNF-R.

One such ligand includes the amino acid sequence for the CDR region of the heavy chain of monoclonal antibody no. 32, shown in FIG. **11** (SEQ ID NO:7), and/or the amino acid sequence for the CDR region of the light chain of this antibody shown in FIG. **12** (SEQ ID NO:11).

Another such ligand includes the amino acid sequence for the CDR region of the heavy chain of monoclonal antibody no. 70 (SEQ ID NO:5) shown in FIG. **11**.

Yet another such ligand includes the amino acid sequence for the CDR region of the heavy chain of monoclonal antibody no. 57 (SEQ ID NO:9), shown in FIG. **11**.

The above antibodies are called herein, for simplicity's sake, "group 32" antibodies.

In another aspect of the invention, the ligands comprise the scFv of a group 32 antibody.

The ligands may comprise e.g. proteins, peptides, immunoadhesins, antibodies or other organic compounds.

The proteins may comprise, for example, a fusion protein of the ligand with another protein, optionally linked by a peptide linker. Such a fusion protein can increase the retention time of the ligand in the body, and thus may even allow the ligand-protein complex to be employed as a latent agent or as a vaccine.

The term "proteins" includes muteins and fused proteins, their salts, functional derivatives and active fractions.

The peptides include peptide bond replacements and/or peptide mimetics, i.e. pseudopeptides, as known in the art (see e.g. Proceedings of the 20th European Peptide Symposium, ed. G. Jung, E. Bayer, pp. 289–336, and references therein), as well as salts and pharmaceutical preparations and/or formulations which render the bioactive peptide(s) particularly suitable for oral, topical, nasal spray, ocular pulmonary, I.V. or subcutaneous delivery, depending on the particular treatment indicated. Such salts, formulations, amino acid replacements and pseudopeptide structures may be necessary and desirable to enhance the stability, formulation, deliverability (e.g. slow release, prodrugs), or to improve the economy of production, as long as they do not adversely affect the biological activity of the peptide.

Besides substitutions, three particular forms of peptide mimetic and/or analogue structures of particular relevance when designating bioactive peptides, which have to bind to a receptor while risking the degradation by proteinases and peptidases in the blood, tissues and elsewhere, may be mentioned specifically, illustrated by the following examples: Firstly, the inversion of backbone chiral centres leading to D-amino acid residue structures may, particularly at the N-terminus, lead to enhanced stability for proteolytical degradation without adversely affecting activity. An example is given in the paper "Tritriated D-ala^1-Peptide T Binding", Smith C. S. et al., Drug Development Res. 15, pp. 371–379 (1988). Secondly, cyclic structure for stability, such as N to C interchain imides and lactames (Ede et al. in Smith and Rivier (Eds.) "Peptides: Chemistry and Biology", Escom, Leiden (1991), pp. 268–270), and sometimes also receptor binding may be enhanced by forming cyclic analogues. An example of this is given in conformationally restricted thymopentin-like compounds", U.S. Pat. No. 4,457,489 (1985), Goldstein, G. et al. Thirdly, the introduction of ketomethylene, methylsulfide or retroinverse bonds to replace peptide bonds, i.e. the interchange of the CO and NH moieties are likely to enhance both stability and potency. An example of this type is given in the paper "Biologically active retroinverso analogues of thymopentin", Sisto A. et al in Rivier, J. E. and Marshall, G. R. (eds) "Peptides, Chemistry, Structure and Biology", Escom, Leiden (1090), pp. 722–773).

The peptides of the invention can be synthesized by various methods which are known in principle, namely by chemical coupling methods (cf. Wunsch, E: "Methoden der organischen Chemie", Volume 15, Band 1+2, Synthese von Peptiden, thime Verlag, Stuttgart (1974), and Barrany, G.; Marrifield, R. B.: "The Peptides", eds. E. Gross, J. Meienhofer, Volume 2, Chapter 1, pp. 1–284, Academic Press (1980)), or by enzymatic coupling methods (cf. Widmer, F. Johansen, J. T., Carlsberg Res. Commun., Vol. 44, pp. 37–46 (1979), and Kullmann, W.: "Enzymatic Peptide Synthesis" CRC Press Inc. Boca Raton, Fla. (1987), and Widmer, F., Johansen, J. T. in "Synthetic Peptides in Biology and Medicines:, eds. Alitalo, K., Partanen, P., Vatieri, A., pp.79–86, Elsevier, Amsterdam (1985)), or by a combination of chemical and enzymatic methods if this is advantageous for the process design and economy.

A cysteine residue may be added at both the amino and carboxy terminals of the peptide, which will allow the cyclisation of the peptide by the formation of a di-sulphide bond.

Any modifications to the peptides of the present invention which do not result in a decrease in biological activity are within the scope of the present invention.

There are numerous examples which illustrate the ability of anti-idiotypic antibodies (anti-Id Abs) to an antigen to function like that antigen in its interaction with animal cells and components of cells. Thus, anti-Id Abs to a peptide hormone antigen can have hormone-like activity and interact specifically with a mediator in the same way as the receptor does. (For a review of these properties see: Gaulton, G. N. and Greane, M. I. 1986. Idiotypic mimicry of biological receptors, Ann. Rev. Immunol. Vol. 4, pp. 253–280; Sege K. and Peterson, P. A., 1978, Use of anti-idiotypic antibodies as cell surface receptor probes, Proc. Natl. Acad. Sci. U.S.A., Vol. 75, pp. 2443–2447).

It is expected from this functional similarity of anti-Id Ab and antigen, that anti-Id Abs bearing the internal image of an antigen can induce immunity to such an antigen. (See review in Hiernaux, J. R., 1988, Idiotypic vaccines and infectious diseases, Infect. Immun., Vol. 56, pp. 1407–1413).

It is therefore possible to produce anti-idiotypic antibodies to the peptides of the present invention which will have similar biological activity.

Accordingly, the present invention also provides anti-idiotypic antibodies to the peptides of the present invention, the anti-idiotypic antibody being capable of inhibiting TNF toxicity, but not its binding to the receptor.

The individual specificity of antibodies resides in the structures of the peptide loops making up the Complementary Determining Regions (CDRS) of the variable domains of the antibodies. Since in general the amino acid sequence of the CDR peptides of an anti-Id Ab are not identical to or even similar to the amino acid sequence of the peptide antigen from which it was originally derived, it follows that peptides whose amino acid sequence in quite dissimilar, in certain contexts, can take up a very similar three-dimensional structure. The concept of this type of peptide, termed a "functionally equivalent sequence" or mimotope by Geyson is known. (Geyson, H. X. et al, 1987, Strategies for epitope analysis using peptide synthesis., J. Immun. Methods, Vol. 102, pp. 259–274).

Moreover, the three-dimensional structure and function of the biologically active peptides can be simulated by other compounds, some not even peptidic in nature, but which nevertheless mimic the activity of such peptides. This field is summarized in a review by Goodman, M. (1990), (Synthesis, Spectroscopy and computer simulations in peptide research, Proc. 11th American Peptide Symposium published in *Peptides-Chemistry, Structure and Biology,* pp. 3–29; Eds. Rivier, J. E. and Marshall, G. R. Publisher Escom).

It is also possible to produce peptide and non-peptide compounds having the same three-dimensional structure as the peptides of the present invention. These "functionally equivalent structures" or "peptide mimics" will react with antibodies raised against the peptide of the present invention and may also be capable of inhibiting TNF toxicity.

Accordingly, a further embodiment of the present invention provides a compound the three-dimensional structure of which is similar as a pharmacophore to the three-dimensional structure of the peptides of the present invention, the compound being characterized in that it reacts with antibodies raised against the peptides of the present invention and that the compound is capable of inhibiting TNF toxicity.

More detail regarding pharmacophores can be found in Bolin a al., p. 150, Polinsky et al., p. 287, and Smith et al., p. 485, in Smith and Rivier (eds.) "Peptides: Chemistry and Biology", Escom, Leiden (1991).

All of the molecules (proteins, peptides, etc.) may be produced either by conventional chemical methods, as described herein, or by recombinant DNA methods.

The invention also provides DNA molecules encoding the ligands according to the invention, vectors containing them and hose cells comprising the vectors and capable of expressing the ligands according to the invention.

The host cell may be either prokaryotic or eukaryotic.

The invention further provides DNA molecules hybridizing to the above DNA molecules and encoding ligands having the same activity.

The invention also provides pharmaceutical compositions comprising the above ligands which are useful for treating diseases induced or caused by the effects of TNF, either endogenously produced or exogenously administered.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows a diagrammatic illustration of the bacterial constructs used for determining the sequence to which antibodies of the 32 group bind. The residues numbered 3 to 235 correspond to residues 25 to 257 of SEQ ID NO:3,

FIG. 2 shows an example of the Western blotting analysis technique by which the binding of the antibodies to the constructs shown in FIG. 1 have been determined.

FIGS. 3 & 4 show the competition of synthetic peptides whose sequences contain the region of the epitope recognized by the monoclonal antibodies of the 32 group, or parts of it, with the binding of an antibody of this group to a construct comprising part of TBP-II in which this epitope is present.

FIG. 5 shows the nucleotide (SEQ ID NO:2) and deduced amino acid (SEQ ID NO:3) sequences of the p75 receptor. TBP-II and transmembranal domains are boxed and shaded. The region recognized by the group 32 antibodies is underlined.

FIG. 6 shows the pattern of protection of HeLa p75.3 cells (as hereinafter defined) from TNF cytotoxicity by different monoclonal antibodies against p75 TNF-R, and fragments thereof.

FIG. 7 shows the effects of a monoclonal antibody against TBP-I and several against TBP-II on the extent of killing of U937 cells by TNF.

FIGS. 8*a* and 8*b* (hereinafter collectively referred to as FIG. 8) show the effects of monoclonal antibody 70 and Fab fragments thereof on the binding of TNF to HeLa p75.3 calls and U937 cells, respectively.

FIGS. 9A–F (hereinafter collectively referred to as FIG. 9) show comparisons of the effects of the antibody 32 with other antibodies against the p75 TNF-R on TNF binding to HeLa p75.3 cells.

FIG. 10 shows dissociation of TNF from HeLa p75.3 cells; namely MoAb #14 (FIG. 9A), MoAb #32 (FIG. 9B), MoAb #31 (FIG. 9C), MoAb #67 (FIG. 9D), MoAb #36 (FIG. 9E) and Polyanti-stalk Ab (FIG. 9F) in the presence and absence of antibody no. 70 and its monovalent Fab fragment.

FIG. 11 shows the nucleotide (SEQ ID NO:4 for #70; SEQ ID NO:6 for #32; SEQ ID NO:8 for #57) and deduced amino acid (SEQ ID NO:5 for #70; SEQ ID NO:7 for #32; SEQ ID NO:9 for #57) sequences for the CDR region of the heavy chains of three monoclonal antibodies of the 32 group.

FIG. 12 shows the nucleotide (SEQ ID NO:10) and deduced amino acid (SEQ ID NO:11) sequences for the CDR region of the light chains of monoclonal antibody No. 32.

FIG. 13 shows the amino acid sequence homology between several members of the TNF/NGF receptor family (residues 3-155 of hu p55 TNF-R (SEQ ID NO:12); residues 39-201 of hu p75 TNF-R (SEQ ID NO:13); residues 31-149 of hu FAS (SEQ ID NO:14); residues 3-161 of hu NGF-R (SEQ ID NO:15); residues 25-187 of hu CDw40 (SEQ ID NO:16); and residues 25-164 of rat Ox40 (SEQ ID NO:17)).

DETAILED DESCRIPTION OF THE INVENTION

TNF, as stated above, is a cytokine which initiates its effect on cell function by binding to two specific call surface receptors: the p55 and p75 receptors. Binding of antibodies to the extracellular domain of these receptors can interfere with its effect. However, as shown in a number of studies, antibodies binding to the extracellular domain of the receptors can also trigger the effects of TNF by inducing aggregation of the p55 receptors, as well as by inducing aggregation of the p75 receptors. (Engelmann, et al. J. Biol. Chem., Vo. 265, No. 24, pp. 14497–14504, 1990; and unpublished data).

The invention relates to antibodies against TBP-II and to F(ab) fragments thereof, and to salts, functional derivatives and/or active fractions (as defined in patent application Ser. No. 07/930,443) thereof. These antibodies provide a new approach for the modulation of the TNF activity, and may be used both to inhibit and to mimic effects of TNF on specific subsets of cells, depending on the molecular form of the antibodies, specifically on their valence: monovalent forms of the antibodies (e.g. F(ab) fragments) being inhibitory and multivalent forms being able to mimic at least part of the effects of TNF. They are, thus, suitable as pharmaceutical agents both for mimicking and blocking TNF effects on cells.

The functional interaction of the antibodies of the present invention with TBP-II provides also a new diagnostic tool, based on immunoassays such as radioimmunoassay, ELISA etc., for the detection of over- or under-production of TBP-II by cells in the body in certain disorders. Thus, the level of TBP-II in sera of patients with different types of cancer or suffering from autoimmune disorders, such as systemic lupus erythematosus (SLE), can be determined this way. In an inverse approach, antibodies against TBP-II, when produced endogenously in the body, will be measured with the use of purified TBP-II. Detecting such autoantibodies, when formed in certain autoimmune disorders, is of extreme importance, since their ability to mimic or inhibit the effects of TNF surely has far-reaching bearing on the pathological syndromes of said disorders.

The antibodies may be either polyclonal or monoclonal. They may be raised in rabbits, mice or other animals or tissue cultured cells derived thereof or can be products of cells of human origin. They may also be produced by recombinant DNA technology either in a form identical to that of the native antibody or as chimeric molecules, constructed by recombination of antibody molecules of man and animal origins or in other forms chosen to make the antibodies most suitable for use in therapy.

For the preparation of the antibodies, either purified TBP-II or one or more synthetic peptides identical to the known sequence of a fragment thereof, e.g. to the N-terminal protein sequence, may be used to immunize animals. A further possibility is to fuse one of the possible nucleotide sequences coding for a fragment of TBP-II to the gene coding for Protein A, to express the fused Protein A-TBP-II gene in *E. coli,* to purify the fused protein by affinity chromatography on IgG SEPHAROSE (beaded agarose gel filtration matrix with broad fractionation range and high exclusion limits for the separation of biomolecules; Pharmacia) column and then to use it to immunize animals.

The monoclonal antibodies of the present invention are prepared using conventional hybridoma technique (Kohler et al. (1975) Nature 256:495; Kohler et al. (1976) Eur. J. Immunol. 6:511). After immunization, spleen cells alone or together with lymph node cells of the immunized animals are isolated and fused with a suitable myeloma cell line. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium and then cloned. The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding

TBP-II. After identification, the desired clones are grown in bulk, either in suspension culture or in ascitic fluid, by injecting the cells into the peritoneum of suitable host mice. The monoclonal antibodies produced by the hybridomas are then isolated and purified.

As mentioned before, the monoclonal antibodies may also be immobilized and used for the purification of the TBP-II in affinity purification procedure using an immunoadsorbent column.

We have found that certain antibodies binding to one particular region in the p75 receptor are not mimetic but rather inhibitory to the signalling for the cytocidal effect by this receptor. This, in spite of the fact that when binding to this region, these antibodies do not block TNF binding, but rather increase it to some extent.

The present invention reveals that this region recognized by these antibodies which we call the 32 group, is the region extending between the two C-terminal cysteines in the extracellular domain of the p75 receptor, plus an additional amino acid, thr179. This region for simplicity's sake, is called "cysteine loop" throughout this specification.

The present invention also provides the nucleotide sequences and deduced amino acid sequences in the CDR of the heavy chain of the three antibodies belonging to this group, named 32, 57 and 70. A remarkable similarity between the sequence of amino acids in the CDR of the heavy chain of the 32 and 70 antibodies was found, indicating that the sequence of amino acids in the CDR of the heavy chain of these two antibodies is close to the optimum necessary for binding to the antigen. In addition, the invention also provides the nucleotide sequence and the deduced amino acid sequence of the light chain of antibody 32. Based on these sequences, small molecular weight compounds, peptides or mimetic compounds which will inhibit the function of the p75 receptors can be defined.

In evidence that such small compounds can indeed achieve this and that there is no need for aggregation of receptors, which antibodies are known to be able to do, it was found that also F(ab) monovalent fragments of the antibodies of the 32 group inhibit signalling for toxicity by the p75 receptor when they are triggered by TNF.

In view of these findings, as well as the close similarity of the receptors in this particular family, this invention relates also to agents which bind to the C-terminal cysteine loop in the extracellular domain of the various other members of the TNF/NGF receptor family and modulate the function of the other receptors, similarly to the modulation of the function of TNF. In this receptor family, the localization of cysteine in the extracellular domain and the spacing is highly conserved. Certain members of this family, e.g. CDw40, exhibit particularly high similarity to the p75 receptor. Particularly in such receptors, agents binding to these regions are expected to have effects similar to the effect of the 32 antibodies on the p75 receptor.

As stated above, the ligands according to the invention may comprise proteins, peptides, immunoadhesins, antibodies or other organic compounds.

Proteins may be isolated from cellular extracts, e.g. by ligand affinity purification employing a molecule having an amino acid sequence substantially corresponding to the above-mentioned stretch as ligand.

Peptides may be prepared by synthetizing first target peptides which correspond to the amino acid stretch of the TNF-R found in accordance with the invention to bind the ligands which inhibit the effects of TNF. Thereafter, peptide libraries are screened for other ligands which bind thereto. The peptides which bind to these regions are further screened for those which also bind to TNF-R. Finally, the peptides capably of high affinity binding with both the target peptides and the TNF-R, are screened for the ability of the peptide to perform the desired biological activity.

In a similar manner, a variety of organic molecules, including drugs known for other indications, are screened for their ability to bind to the amino acid stretch found to be critical for inhibiting the effects of TNF.

In addition to the organic molecules, also broth of biological matter such as bacteria culture products, fungi culture products, eukaryotic culture products and crude cytokine preparations are screened with the amino acid target peptides described above. Molecules obtained by this screening are then further screened for their ability to perform the desired biological function.

Alternatively, molecules are designed which spatially fit the quaternary structure of the amino acid stretch in the receptor.

The active molecules obtained by the above procedures, inasfar as they are biological substances, can also be prepared by biotechnological approaches. In this way, massive production of these molecules will be made possible. Peptides may either be produced by known peptide synthesis methods or using expression vectors containing DNA sequences encoding them. Other molecules, if produced in an enzymatic way, can be made by producing the enzymes involved in the appropriate cultured cells.

Pharmaceutical compositions containing the ligands of the present invention may be employed for antagonizing the effects of TNF in mammals.

Such compositions comprise the ligands according to the invention as their active ingredient. The pharmaceutical compositions are indicated for conditions such as septic shock, cachexia, graft-versus-host reactions, autoimmune diseases such as rheumatoid arthritis, and the like. They are also indicated for counteracting e.g. an overdose of exogenously administered TNF.

The pharmaceutical compositions according to the invention are administered depending on the condition to be treated, via the accepted ways of administration. For example, in the case of septic shock, intravenous administration will be preferred. The pharmaceutical compositions may also be administered continuously, i.e. by way of infusion, or orally. The formulation and dose will depend on the condition to be treated, the route or administration and the condition and the body weight of the patient to be treated. The exact dose will be determined by the attending physician.

The pharmaceutical compositions according to the invention are prepared in the usual manner, for example by mixing the active ingredient with pharmaceutically and physiologically acceptable carriers and/or stabilizers and/or excipients, as the case may be, and are prepared in dosage form, e.g. by lyophilization in dosage vials.

As used herein the term "muteins" refers to analogs of the proteins, peptides and the like in which one or more of the amino acid residues of the protein found to bind are replaced by different amino acid residues or are deleted, or one or more amino acid residues are added to the original sequence, without changing considerably the activity of the resulting product. These muteins are prepared by known synthesis and/or by site-directed mutagenesis techniques, or any other known technique suitable therefor.

The term "fused protein" refers to a polypeptide comprising the ligands or a mutein thereof fused with another

protein which has an extended residence time in body fluids. The ligands may thus be fused to another protein, polypeptide or the like, e.g. an immunoglobulin or a fragment thereof.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of the ligands, muteins and fused proteins thereof. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids such as, for example, acetic acid or oxalic acid.

"Functional derivatives" as used herein cover derivatives of the ligands and their fused proteins and muteins, which may be prepared from the functional groups which occur as side chains on the residues or the N- or C- terminal groups, by means know in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e. they do not destroy the activity of the ligand and do not confer toxic properties on compositions containing it. These derivatives may, for example, include polyethylene glycol sidechains which may mask antigenic sites and extend the residence of the ligands in body fluids. Other derivatives include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl moieties (e.g. alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl or threonyl residues) formed with acyl moieties.

The invention is illustrated by the following non-limiting examples:

EXAMPLE 1

Monoclonal Antibodies to TBP-II Production of the Monoclonal Antibodies

Female Balb/C mice (8 weeks old) were injected with 1 μg purified TBP-II in an emulsion of complete Freund's adjuvant into the hind foot pads, and three weeks later, subcutaneously into the back in incomplete Freund's adjuvant. The other injections were given in weekly intervals, subcutaneously in PBS. Final boosts were given 4 days (i.p.) and 3 days (i.v.) before the fusion with 9.0 μg of TBP-I in PBS. Fusion was performed using NSO/Mr cells and lymphocytes prepared from both the spleen and the local lymphocytes of the hind legs as fusion partners. The hybridomas were selected in DMEM supplemented with HAT, 15% horse serum and gentamycin 2 μg/ml. Hybridomas that were found to produce antibodies to TBP-I were subcloned by the limiting dilution method and injected into Balb/C mice that had been primed with pristane for the production of ascites. Immunoglobulins were isolated from the ascites by ammonium sulfate precipitation (50% saturation) and then dialyzed against PBS containing 0.02% azide. Purity was approximately 60% as estimated by analysis on SDS-PAGE and staining with Commassie blue. The isotypes of the antibodies were defined with the use of a commercially available ELISA kit (Amersham, U.K.).

Several positive clones were obtained, subcloned for further studies and characterized. Some of the isolated subclones with their isotype and binding of TBP-II in inverted RIA are listed in Table I.

TABLE I

Subclones producing monoclonal antibodies to TBP-II

Clone number	Screening with iRIA [CPM]	Screening of subclone with iRIA [CPM]	Isotype
13.11	31800	31000	IgG_1
.12		31500	IgG_1
.13		31100	IgG_1
14.1	15300	15400	IgG_{2a}
.6		16200	IgG_{2a}
.7		15300	IgG_{2a}
20.2	12800	14200	IgG_{2b}
.5		14300	IgG_{2b}
.6		14800	IgG_{2b}
22.7	20400	20000	IgG_1
.8		19300	IgG_1
27.1	18000	27000	IgG_{2a}
.3		25000	IgG_{2a}
.9		28000	IgG_{2a}
32.4	11315	10900	IgG_{2b}
.5		10700	IgG_{2b}
.6		11200	IgG_{2b}
33.1	18400	11400	IgG_1
.3		10500	IgG_1
.4		14800	IgG_1
36.1	27500	26600	IgG_{2a}
.5		24900	IgG_{2a}
.6		24900	IgG_{2a}
41.3	13800	18100	IgG_1
.7		18100	IgG_1
.10		18800	IgG_1
67.1	16800	10900	IgG_{2a}
.16		10800	IgG_{2a}
.17		10900	IgG_{2a}
70.2	15100	5100	IgG_{2a}
.3		5200	IgG_{2a}
.4		5300	IgG_{2a}
77.2	15300	11800	IgG_{2b}
78.9	25300	21400	IgG_{2a}
82.1	17600	25900	IgG_1
.4		25700	IgG_1
.10		26400	IgG_1
86.2	8800	12200	IgG_{2b}
.5		12600	IgG_{2b}
.11		12800	IgG_{2b}
19.6		29700	IgG_{2a}
.9		28900	IgG_{2a}

Hybridomas TBP-II 13-12 and TBP-II 70-2 were deposited with the Collection Nationale de Cultures de Microorganismes (CNCM), Institut Pasteur, 25, rue du Docteur Roux, 75724 Paris CEDEX 15, France on Mar. 12, 1990 and were assigned No. I-929 and No. I-928, respectively. Hybridoma 32-5 was deposited with the CNCM on Sep. 1, 1993, and assigned No. I-1358. Another clone producing monoclonal antibodies to TBP-II is hybridoma 57-1, which was deposited with the CNCM on Apr. 23, 1996, and assigned No. I-1696.

EXAMPLE 2

Inverted Radioimmunoassay (iRIA) for the Detection of the Monoclonal Antibodies to TBP-II

This assay was used for estimating the level of the anti-TBP antibodies in the sera of the immunized mice and for screening for the production of the antibodies by hybridomas. PVC, 96-well microtiter plates (Dynatech 1-220-25) were coated for 12 hr at 4° C. with affinity purified goat anti mouse F(ab) immunoglobulins (Biomaker, Israel 10 μg/ml in PBS containing 0.02% NaN_3), then blocked for 2 hr at 37°

C. with 0.5% BSA in PBS supplemented with 0.05% TWEEN 20 (polyoxyethylene sorbitan monolaurate; Sigma) and 0.02% NaN_3 (blocking buffer) and washed 3 times with PBS containing 0.05% Tween 20 and 0.02% NaN_3 (washing buffer). Serum samples, in serial dilutions, or samples of hybridoma growth media (50 µl) were applied into the wells for 2 hr at 37° C. The plates were rinsed with washing buffer and ^{125}I-labelled TBP-I (10,000 cpm, in blocking buffer) was applied into the wells. After further incubation of 2 hr at 37° C., the plates were washed and the amount of label which bound to individual wells was determined in the gamma-counter.

EXAMPLE 3

The Use of Anti-TBP-II Antibodies for Affinity Chromatography

Antibodies against TBP-II can be utilized for the purification of TBP-II by affinity chromatography, according to the following procedure. The monoclonal antibodies for affinity chromatography were selected by testing their binding capacity for the radiolabeled antigen in a solid phase radio immunoassay. Ascites from all hybridomas was purified by amonium sulfate precipitation at 50% saturation followed by extensive dialysis against PBS. PVC 96-well plates were coated with the purified McAbs, and after blocking the plates with PBS containing 0.5% BSA, 0.05% TWEEN 20 (Sigma) and 0.02% NaN_3, the wells were incubated with 50,000 cpm ^{125}I-TNF for 2 h at 37° C., then washed and the radioactivity which had bound to each well was quantitated in the gamma-counter. The antibodies with the highest binding capacity were examined for their performance in immunoaffinity chromatography.

Polyacryl hydrazide agarose was used as resin to immobilize the antibodies. The semipurified immunoglobulins were concentrated and coupled to the resin as specified by Wilchek and Miron, *Methods in Enzymology* 34:72–76, 1979. Three monoclonal antibodies against TBP-I, clones 16, 20, and 34 were tested in these experiments. Antibody columns of 1 ml bed were constructed. Before use, all columns were subjected to 10 washes with the elution buffer, each wash followed by neutralization with PBS. Then the columns were loaded with 120 ml of concentrated urinary proteins in PBS with 0.02% NaN_3. The flow rate of the columns was adjusted to 0.2 to 0.3 ml per minute. After loading, the columns were washed with 50 ml PBS and then eluted with a solution containing 50 mM citric acid, pH 2.5, 100 mM NaCl and 0.022 NaN_3. Fractions of 1 ml were collected. Samples of the applied urinary proteins, the last portion of the wash (1 ml) and of each elution fraction (8 fractions of 1 ml per column) were taken and tested for protein concentration and activity in the bioassay for TBP-II. According to the protein measurements before and after coupling of the antibodies to hydrazide agarose, the amounts of immunoglobulin bound to the columns ranged from 7 to 10 mg/ml agarose. All protein measurements were done according to a micro-flurescamin method in comparison to a standard solution containing 100 µg BSA/ml (Stein, S. and Moschera, J., *Methods Enzymol.* 79:7–16, 1981).

EXAMPLE 4

Determination of TBP-II Using Anti-TBP-II Antibodies

The levels of TBP-II in the sera of healthy individuals, patients with cancer or systemic lupus erthematosus (SLE) and of pregnant women at term were determined by an ELISA method employing a monoclonal antibody to TBP-II coating the plates. 50 µl of each sample was added and after a 2.5 h incubation at 37° C. the wells were washed with a solution of PBS, Tween 0.05% and sodium azide 0.02%, after which a rabbit anti-TBP-II polyclonal antibody was added for 2.5 h at 37° C. Then the wells were washed again (no azide) and goat anti-rabbit horseradish peroxidase-coupled antibody was added for 2 h. Following this incubation, and washing, an ABTS buffer was added and optical density (O.D.) read 30 min. later at 600 nm.

The normal levels of TBP-II in human serum of healthy individuals as determined by the ELISA method are 1.48±0.46 ng/ml.

EXAMPLE 5

Epitope Mapping of TBP-II by Cross Competition Analysis with Monoclonal Antibodies (mAbs) to TBP-II

PVC 96-well microtiter plates were coated as described above, with purified mAbs to TBP-II (25 µg/ml). Following rinsing and blocking, samples of ^{125}I-labelled TBP-II (100,000 cpm per well) which had been preincubated for 2 h, at 37° C. with the same or a different monoclonal antibody to TBP-II (at 1 µg/ml) were put into the wells; the plates were incubated overnight at 4° C., washed and the radioactivity bound to each well was determined by gamma-counting. The results are expressed as percent of the control values (TBP-II binding in the absence of competing mAbs).

The results are depicted in Table II. The monoclonal antibodies are indicated by the clone numbers in the first raw and in left column. Low percent binding values indicate that the two antibodies compete for each other's epitope on TBP-II, while higher values indicate that they bind to different epitopes. Non-competitive antibodies are suitable for use in double-sandwich ELISA, e.g., clones 13 and 70.

TABLE II

	Cross competition analysis with monoclonal antibodies to TBP II															
competitor	solid phase antibodies															
antibody	13	14	19	20	22	27	32	33	36	41	67	70	77	78	82	86
13	4	64	53	73	31	51	161	35	177	72	131	128	77	102	50	101
14	119	20	90	13	13	84	156	11	132	173	134	113	14	70	89	179
19	103	28	7	19	11	5	144	11	144	133	179	123	18	5	85	126
20	119	17	93	14	10	88	149	9	135	170	137	135	16	70	101	181
22	109	26	94	22	13	82	128	12	115	164	136	114	17	68	98	167
27	106	23	11	27	14	8	145	17	152	133	196	136	24	8	82	125

TABLE II-continued

Cross competition analysis with monoclonal antibodies to TBP II

competitor	solid phase antibodies															
antibody	13	14	19	20	22	27	32	33	36	41	67	70	77	78	82	86
32	150	267	150	291	156	186	14	163	139	200	205	18	294	143	103	226
33	115	19	98	23	16	86	133	12	118	156	120	114	24	78	90	155
36	155	262	168	271	144	185	167	158	12	169	223	135	265	158	93	150
41	117	119	119	118	101	109	118	76	93	9	179	107	106	111	8	9
67	112	138	125	141	125	157	136	107	138	213	30	117	120	127	106	236
70	150	246	150	255	145	166	4	162	166	217	204	6	232	132	107	234
77	121	18	98	15	13	78	148	11	145	184	142	132	18	66	103	184
78	118	20	9	26	10	6	153	13	157	137	183	131	19	6	94	172
82	107	110	130	116	112	121	128	89	90	8	162	102	121	113	8	7
86	122	181	125	166	126	129	131	120	86	18	253	109	152	125	20	17
100% value	31582	3958	2057	5437	4947	17395	25923	3525	6368	8042	4368	24113	5887	22222	11608	9703

EXAMPLE 6

Determination of the Region of the p75 Receptor Which is Recognized by the Group 32 Antibodies

We have now prepared a number of constructs and the complete list of constructs examined, as well as their relationship to the structure of the soluble p75R are shown in FIG. 1. Constructs recognized by the antibodies of the 32 group are listed in bold numbers and illustrated as solid lines. Those not reacting with these antibodies are listed in thin numbers and illustrated by broken lines. All constructs are identified by their N- and C-terminal amino acid residues. It can therefore be concluded that the epitope recognized by antibody no. 32 maps between amino acids 163–179, which corresponds to residues 185–201 of SEQ ID NO:3.

FIG. 1, above the diagrammatic illustration of the constructs, shows the amino acid sequence of part of the p75 TNF-R, the regions corresponding to the soluble form of the receptor and the transmembranal region being boxed. Amino acid residues conserved between man and mouse are underlined.

EXAMPLE 7

Competition for Binding to the Extracellular Domain of the p75 TNF-R Between Group 32 Antibodies and Synthetic Peptides

A number of synthetic peptides whose sequences correspond to various parts of the region on the TNF-R suspected to be the group 32 epitope were synthetized (residues 160-179, 162-179, 163-179, 165-179 and 167-179) corresponding to residues 182-201, 184-201, 185-201, 187-201 and 189-201 of SEQ ID NO:3, respectively). The peptides were examined in an ELISA test for their ability to compete for the binding to the antibodies of the 32 group.

A bacterially produced construct corresponding to amino acids 3 to 180 of the p75 TNF-R (p75 construct in FIG. 3 corresponding to 25 to 202 of SEQ ID NO:3)) was applied, at the indicated concentrations, to PVC plates precoated with antibody 32 followed by application of rabbit antiserum to TBP-II (p75 soluble TNF-R). The amount of rabbit antiserum bound to the plate was determined by applying goat antiserum against rabbit immunoglobulin, coupled to horse-radish peroxidase and enzymatic assessment of the amount of goat immunoglobulin bound to the plate. FIG. 3 shows the data of an experiment in which a synthetic peptide corresponding to amino acid residues 163 to 179 was found to compete for the binding.

FIG. 4 shows the data of an experiment in which a fusion protein of maltose binding protein (MBP) with the sequence of amino acids extending from 125 to 192 of the p75 (corresponding to residues 147-214 of SEQ ID NO:3) receptor was used to coat PVC plates at a concentration of 10 μg/ml, then the No. 32 McAb was applied at a concentration of 2 μg/ml together with the indicated concentrations of different peptides:

DW16—amino acids 165-179 (corresponding to residues 187 to 201 of SEQ ID NO:3)

DW18—amino acids 163-179 (corresponding to residues 185 to 201 of SEQ ID NO:3)

DW19—amino acids 162-179 (corresponding to residues 184 to 201 of SEQ ID NO:3)

DW21—amino acids 160-179 (corresponding to residues 182 to 201 of SEQ ID NO:3)

Thereafter, the reaction was developed by adding goat anti-mouse coupled to horseradish peroxidase. As shown in FIG. 4, marked inhibition of fusion protein recognition by monoclonal antibody No. 32 was observed only with the three peptides covering the whole epitope.

EXAMPLE 8

Mutational Study of the 32 Epitope

Replacing cysteine 178 with alanine in a recombinant peptide whose sequence corresponds to amino acids 3 to 181 (SEQ ID NO:5), made this protein unrecognizable by the 32 group antibodies. This finding suggests that in order to be recognized by these antibodies, the two cysteines in the group 32 epitope region must be free to interact with each other; i.e. that the structure recognized by the antibodies is a loop. In support of this notion, we found that reduction of the peptide with dithiothreithol prior to SDS PAGE and Western blotting analysis somewhat decreased the effectivity of its recognition by the 32 group antibodies, and reduction by dithiothreithol followed by alkylation with iodoacatimide made it completely unrecognizable by the antibodies.

EXAMPLE 9

Effects of Various Antibodies and Fragments Thereof on TNF Toxicity

(a) In order to compare the function of the 32 group antibodies, not only to antibodies which bind to the

receptor upstream to the 32 epitope region (as most of the anti-TBP-II antibodies are expected to), but also to antibodies that bind to the receptor downstream to that epitope region, we immunized mice with a chimeric construct corresponding to the region extending downstream to the 32 epitope (amino acids 181 to 235 which corresponds to residues 203 to 257 of SEQ ID NO:3; the "stalk" region), linked to MBP. The rabbits developed antibodies which bound to the chimera with which they were immunized as well as to the intact p55 TNF receptor. These antibodies were affinity purified by binding to the chimeric protein, linked to an AFFI-GEL 10 column (crosslinked agarose matrix with N-hydroxysuccinimide as functional group; BioRad), and tested for effect on TNF function and binding. (The affinity purified antibody preparation was termed "318").

(b) All monoclonal anti-TBP-II antibodies as well as the affinity purified antistalk antibodies were tested for effect on TNF toxicity in clones of the epitheloid HeLa cells which were made to over-express the p75 receptors (by their transfection with the p75 receptor's cDNA. We called the particular over-expressing clone used in the experiments presented here, HeLa p75.3). The only antibodies found to inhibit TNF function were the antibodies of the group 32 epitope; that, in spite of the fact that they do not inhibit, but somewhat increase TNF binding to the receptor (FIGS. **8** and **9**). Two of the other anti-TBP-II antibodies (No. 67 of FIGS. **6** and **9** and number 81) had very little effect on TNF binding to the receptor or on TNF toxicity. All other monoclonal anti-TBP-II antibodies somewhat potentiated the cytocidal effect of TNF even though competing with TNF binding (e.g. antibody 36 of FIGS. **6** and **9**). The "anti-stalk" antibodies had very little effect on TNF binding or function (FIGS. **6** and **9**). Applying the anti-stalk antibodies on the cells together with antibodies of the 32 group did not interfere with the inhibitory effect of the latter on TNF function.

(c) The same panel of antibodies was tested for effect on the killing of the myelocytic U937 cells by TNF. As opposed to the mimetic effect of anti-TNF receptor antibodies in the HeLa cells, neither anti-p55 nor anti-p75 receptor antibodies were found to be mimetic to the cytocidal effect of TNF on the U937 cells under the conditions of the experiment carried out. Having no ability to mimic the effect of TNF, all monoclonal antibodies which compete for TNF binding either to the p75 receptor, (e.g. antibodies 14, 31 and 36 of FIG. **9**) or to the p55 receptor (e.g. antibody number 18 of FIG. **7**) are inhibitory to the TNF effects. Antibodies which had no affect on TNF binding to the receptors (e.g. number 67 of FIG. **9**) had no effect on TNF function (FIG. **6**). The 32 group antibodies were unique in having an ability to inhibit TNF function in this cell without having any inhibitor effect on TNF binding. The antibodies actually enhanced the binding of TNF to these cells, much more so than in the HeLa p75.3 cells (FIG. **8**). The inhibitory effect of the 32 group antibodies was additive to that of antibodies which block TNF binding to the p55 receptor (e.g. the combination 18/32 in FIG. **7**).

EXAMPLE 10

Effect of Group 32 Antibodies and Fab Monovalent Fragments Thereof on the Dissociation of TNF from the TNF-Rs

In order to explore the mechanism by which the 32 group antibodies cause an increase in TNF binding, we compared the rate of TNF dissociation from HeLa p75.3 cells in the presence and absence of these antibodies.

Radiolabelled TNF was added to confluent HeLa p75.3 cells and the cells were incubated for 2 hr on ice. Unbound ligand was washed away and 1 ml of binding buffer containing 500 ng/ml of cold TNF was applied into quadruplicate wells for the indicated periods of time on ice. Thereafter, the wells were washed once again with cold PBS, and the amount of residual ligand was determined by measuring radioactivity of cells detached from plates by incubation with PBS/EDTA solution. The antibodies were applied throughout the assay at a concentration of 10 μg/ml.

As illustrated in FIG. **10**, both these antibodies as well as their F(ab) monovalent fragments caused a decrease in the rate of TNT dissociation from the receptors. Besides providing a possible explanation for the way in which these antibodies affect TNF binding to its receptors, this finding indicates an additional application for this effect. Soluble forms of to p75 TNF-Rs or of the p55 receptor or of any other member of the TNF/BGF receptor family in which a conformational change as that imposed by the 32 group antibody will occur, will severe as better inhibitors of the respective agonist.

EXAMPLE 11

Determination of Nucleotide Sequences and Deduced Amino Acid Sequences in the CDR of the Heavy Chains of Monoclonal Antibodies 32, 57 and 70 (Group 32 Antibodies) and in the CDR of the Light (Kappa) Chain of Antibody 32

In order to determine the nucleotide sequences of the CDR of the heavy chains of antibodies 32, 57 and 70, total RNA was isolated by the Promega protocol from the respective hybridoma cells, with the use of guanidinium thioisocyanate. First strand cDNA synthesis on this RNA was performed with the use of AMV reverse transcriptase and either oligo(dT)15-18 or an oligonucleotide complementary to the constant region of the heavy chain of murine IgG as a primer. The cDNA was used as a template for PCR, applying a partially degenerate 5'-Primer. 40 cycles of PCR were carried out. PCR products with the size of about 350 bp were purified electrophoretically and cloned into the Bluescript vector. Clones having inserts of the right size were sequenced. Double-stranded cDNA of the CDR region of the light chain of: antibody no. 32 was synthesized in a similar manner.

The nucleotide sequences obtained by the dideoxy chain termination method, and the amino acid sequences deduced therefrom are shown in FIGS. **11** and **12**. The CDR1, 2 and 3 regions are underlined.

EXAMPLE 12

Preparation of scFv of the 32 Group Antibodies

The cloned variable regions of the heavy and light chains of the monoclonal antibodies of the 32 group are linked with a linker of 15 amino acid length and introduced into a commercial expression vector. The vector contains a promoter, e.g. lac, a leader sequence e.g. pel-B, as well as a sequence encoding a small peptide ("tag" peptide) against which a monoclonal antibody is commercially available. The plasmid is now introduced into *E. coli* and the bacteria are grown to O.D. 0.5–1.0. Expression of scFv is induced by addition of IPTG and growth is continued for another 6–24 hrs. The soluble scFv-tag complex is then isolated from the

culture medium by immunoaffinity purification using the monoclonal antibody against the tag and then purified on a metaloaffinity column.

Any scFv accumulating within the bacteria is purified by isolating and repeatedly washing the inclusion bodies, followed by solublization by e.g. urea or guanidinium and subsequent renaturation.

Alternative possibilities are employing an oligohistidine as the tag, using a stronger promoter instead of lac, i.e. T7, constructing the vector without the leader sequence or introducing a sequence encoding a "tail" of irrelevant sequences into the vector at the 5' end of the scFv. This "tail" should not be biologically active, since its only purpose is the creation of a longer molecule than the native scFv, thus causing a longer retention time in the body.

EXAMPLE 13

FIG. 13 shows the internal cysteine rich repeats in the extracellular domains of the two TNF-Rs and their alignment with the homologous repeats in the extracellular domain of the human FAS, nerve growth factor receptor (NGF) and Cw40, as well as rat Ox40. The amino acid sequences (one letter symbols) are aligned for maximal homology. The positions of the amino acids within the receptors are denoted in the left hand margin.

EXAMPLE 14

Creation of Recombinant DNA Molecules Comprising Nucleotide Sequences Coding for the Active Peptides and Other Molecules and Their Expression

The peptides and other molecules can also be prepared by genetic engineering techniques and their preparation encompasses all the tools used in these techniques. Thus DNA molecules are provided which comprise the nucleotide sequence coding for such peptides and other biological molecules. These DNA molecules can be genomic DNA, cDNA, synthetic DNA and a combination thereof.

Creation of DNA molecules coding for such peptides and molecules is carried out by conventional means, once the amino acid sequence of these peptides and other molecules has been determined.

Expression of the recombinant proteins can be effected in eukaryotic cells, bacteria or yeasts, using the appropriate expression vectors. Any method known in the art may be employed.

For example, the DNA molecules coding for the peptides or other molecules obtained by the above methods are inserted into appropriately constructed expression vectors by techniques well known in the art (see Maniatis, T. et al., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor (1982)). Double-stranded cDNA is linked to plasmid vectors by homopolymeric tailing or by restriction linking involving the use of synthetic DNA linkers or blunt-ended ligation technique. DNA ligases are used to ligate the DNA molecules and undesirable joining is avoided by treatment with alkaline phosphatase.

In order to be capable of expressing a desired biological substance, i.e. a peptide or protein (hereinafter "protein", for simplicity's sake), an expression vector should comprise also specific nucleotide sequences containing transcriptional and translational regulatory information linked to the DNA coding for the desired protein in such a way as to permit gene expression and production of the protein. First, in order for the gene to be transcribed, it must be preceded by a promoter recognizable by RNA polymerase, to which the polymerase binds and thus initiates the transcription process. There are a variety of such promoters in use, which work with different efficiencies (strong and weak promoters). They are different for prokaryotic and eukaryotic cells.

The promoters that can be used in the present invention may be either constitutive, for example, the int promoter of bacteriophage lambda, the bla promoter of the β-lactamase gene of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene of pPR325, etc., or inducible, such as the prokaryotic promoters including the major right and left promoters of bacteriophage lambda (P_L and P_R), the trp, recA, lacZ, lacI, ompF and gal promoters of *E. coli,* or the trp-lac hybrid promoter, etc. (Glick, B. R. (1987) J. Ind. Microbiol., 1:277–282).

Besides the use of strong promoters to generate large quantities of mRNA, in order to achieve high levels of gene expression in prokaryotic cells, it is necessary to use also ribosome-binding sites to ensure that the mRNA is efficiently translated. One example is the Shine-Dalgarno (SD) sequence appropriately positioned from the initiation codon and complementary to the 3'-terminal sequence of 16S RNA.

For eukaryotic hosts, different transcriptional and translational regulatory sequences may be employed, depending on the nature of the host. They may be derived from viral sources, such as adenovirus, bovine papilloma virus, Simian virus, or the like, where the regulatory signals are associated with a particular gene which has a high level of expression. Examples are the TK promoter of Herpes virus, the SV40 early promoter, the yeast gal4 gene promoter, etc. Transcriptional initiation regulatory signals may be selected which allow for repression and activation, so that expression of the genes can be modulated.

The DNA molecule comprising the nucleotide sequence coding for the peptides or other molecules of the invention and the operably linked transcriptional and translational regulatory signals is inserted into a vector which is capable of integrating the desired gene sequences into the host cell chromosome. The cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotropic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of single chain binding protein mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, H., (1983) Mol. Cell Biol., 3:280.

In a preferred embodiment, the introduced DNA molecule will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Preferred prokaryotic vectors include plasmids such as those capable of replication in *E. coli*, for example, pBR322, Co1E1, pSC101, pACYC 184, etc. (see Maniatis et al., (1982) op. cit.); Bacillus plasmids such as pC194, pC221, pT127, etc. (Gryczan, T., *The Molecular Biology of the Bacilli*, Academic Press, NY (1982)); Streptomyces plasmids including pIJ101 (Kendall, K. J. et al., (1987) J. Bacteriol. 1:4177–83); Streptomyces bacteriophages such as ΦC31 (Chater, K. F. et al., in: *Sixth International Symposium on Actinomycetales Biology*, (1986)), and Pseudomonas plasmids (John, J. F., et al. (1986) Rev. Infect. Dis. 8:693–704; and Izaki, K. (1978) Jpn. J. Bacteriol., 33:729–742).

Preferred eukaryotic plasmids include BPV, vaccinia, SV40, 2-micron circle, etc., or their derivatives. Such plasmids are well known in the art (Botstein, D., et al. (1982) Miami Wint. Symp. 19, pp. 265–274; Broach, J. R., in: *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., pp. 445–470 (1981); Broach, J. R., (1982) Cell, 28:203–204; Bollon, D. P., et al. (1980) J. Clin. Hematol. Oncol., 10:39–48; Maniatis, T., in: *Cell Biology: A Comprehensive Treatise, Vol.* 3: *Gene Expression*, Academic Press, NY, pp. 503–608 (1980)).

Once the vector or DNA sequence containing the construct(s) has been prepared for expression, the DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means: transformation, transfection, conjugation, protoplast fusion, electroporation, calcium phosphate-precipitation, direct microinjection, etc.

Host cells to be used in this invention may be either prokaryotic or eukaryotic. Preferred prokaryotic hosts include bacteria such as *E. coli*, Bacillus, Streptomyces, Pseudomonas, Salmonella, Serratia, etc. The most preferred prokaryotic host is *E. coli*.

Bacterial hosts of particular interest include *E. coli* K12 strain 294 (ATCC 31446), *E. coli* X1776 (ATCC 31537), *E. coli* W3110 (F^-, lambda$^-$, prototropic (ATCC 27325)), and other enterobacterium such as *Salmonella typhimurium* or *Serratia marcescens* and various Pseudomonas species. Under such conditions, the protein will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

Preferred eukaryotic hosts are mammalian cells, e.g. human, monkey, mouse and chinese hamster ovary (CHO) cells, because they provide post-translational modifications to protein molecules including correct folding or glycosylation at correct sites. Also yeast cells can carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammal an gene products and secretes peptides bearing leader sequences (i.e. pre-peptides).

After the introduction of the vector, the host cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene sequence(s) results in the production of the desired proteins.

Purification of the recombinant proteins is carried out by any one of the methods known for this purpose.

"Increased" or "substantially" increased inhibition of TNF by a ligand or soluble or mutated soluble TNF/NGF receptor means an increase over a suitable control, within experimental error, of at least one selected from the group consisting of 1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 15, 20, 2,, 30, 35, 40, 45, 50, 100, 200, 300, 400, 500, or 100,000 percent or any range or value therein, such as 1000, 2000, 5000, 10,000, 20,000, 50,000, 100,000%.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 17

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 58 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Ala Gln Val Phe Thr Thr His Gln Ile Cys Asn Val Val Ala Ile Pro
1               5                  10                  15

Gly Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Val
            20                  25                  30

Asp Phe Ala Leu Pro Val Gly Leu Ile Cys Asn Val Val Ala Ile Pro
        35                  40                  45

Gly Asn Ala Ser Met Asp Ala Val Cys Thr
    50                  55
```

-continued

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2224 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 90..1472

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GCGAGCGCAG CGGAGCCTGG AGAGAAGGCG CTGGGCTGCG AGGGCGCGAG GGCGCGAGGG      60

CAGGGGGCAA CCGGACCCCG CCCGCACCC ATG GCG CCC GTC GCC GTC TGG GCC       113
                                Met Ala Pro Val Ala Val Trp Ala
                                  1               5

GCG CTG GCC GTC GGA CTG GAG CTC TGG GCT GCG GCG CAC GCC TTG CCC       161
Ala Leu Ala Val Gly Leu Glu Leu Trp Ala Ala Ala His Ala Leu Pro
     10                  15                  20

GCC CAG GTG GCA TTT ACA CCC TAC GCC CCG GAG CCC GGG AGC ACA TGC       209
Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys
 25                  30                  35                  40

CGG CTC AGA GAA TAC TAT GAC CAG ACA GCT CAG ATG TGC TGC AGC AAA       257
Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys Ser Lys
                 45                  50                  55

TGC TCG CCG GGC CAA CAT GCA AAA GTC TTC TGT ACC AAG ACC TCG GAC       305
Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr Ser Asp
             60                  65                  70

ACC GTG TGT GAC TCC TGT GAG GAC AGC ACA TAC ACC CAG CTC TGG AAC       353
Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu Trp Asn
         75                  80                  85

TGG GTT CCC GAG TGC TTG AGC TGT GGC TCC CGC TGT AGC TCT GAC CAG       401
Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser Asp Gln
     90                  95                  100

GTG GAA ACT CAA GCC TGC ACT CGG GAA CAG AAC CGC ATC TGC ACC TGC       449
Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys Thr Cys
105                 110                 115                 120

AGG CCC GGC TGG TAC TGC GCG CTG AGC AAG CAG GAG GGG TGC CGG CTG       497
Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys Arg Leu
                125                 130                 135

TGC GCG CCG CTG CGC AAG TGC CGC CCG GGC TTC GGC GTG GCC AGA CCA       545
Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala Arg Pro
            140                 145                 150

GGA ACT GAA ACA TCA GAC GTG GTG TGC AAG CCC TGT GCC CCG GGG ACG       593
Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro Gly Thr
        155                 160                 165

TTC TCC AAC ACG ACT TCA TCC ACG GAT ATT TGC AGG CCC CAC CAG ATC       641
Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His Gln Ile
    170                 175                 180

TGT AAC GTG GTG GCC ATC CCT GGG AAT GCA AGC ATG GAT GCA GTC TGC       689
Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala Val Cys
185                 190                 195                 200

ACG TCC ACG TCC CCC ACC CGG AGT ATG GCC CCA GGG GCA GTA CAC TTA       737
Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val His Leu
                205                 210                 215

CCC CAG CCA GTG TCC ACA CGA TCC CAA CAC ACG CAG CCA ACT CCA GAA       785
Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr Pro Glu
            220                 225                 230

CCC AGC ACT GCT CCA AGC ACC TCC TTC CTG CTC CCA ATG GGC CCC AGC       833
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Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly Pro Ser
        235                 240                 245

CCC CCA GCT GAA GGG AGC ACT GGC GAC TTC GCT CTT CCA GTT GGA CTG      881
Pro Pro Ala Glu Gly Ser Thr Gly Asp Phe Ala Leu Pro Val Gly Leu
    250                 255                 260

ATT GTG GGT GTG ACA GCC TTG GGT CTA CTA ATA ATA GGA GTG GTG AAC      929
Ile Val Gly Val Thr Ala Leu Gly Leu Leu Ile Ile Gly Val Val Asn
265                 270                 275                 280

TGT GTC ATC ATG ACC CAG GTG AAA AAG AAG CCC TTG TGC CTG CAG AGA      977
Cys Val Ile Met Thr Gln Val Lys Lys Lys Pro Leu Cys Leu Gln Arg
                285                 290                 295

GAA GCC AAG GTG CCT CAC TTG CCT GCC GAT AAG GCC CGG GGT ACA CAG     1025
Glu Ala Lys Val Pro His Leu Pro Ala Asp Lys Ala Arg Gly Thr Gln
            300                 305                 310

GGC CCC GAG CAG CAG CAC CTG CTG ATC ACA GCG CCG AGC TCC AGC AGC     1073
Gly Pro Glu Gln Gln His Leu Leu Ile Thr Ala Pro Ser Ser Ser Ser
        315                 320                 325

AGC TCC CTG GAG AGC TCG GCC AGT GCG TTG GAC AGA AGG GCG CCC ACT     1121
Ser Ser Leu Glu Ser Ser Ala Ser Ala Leu Asp Arg Arg Ala Pro Thr
    330                 335                 340

CGG AAC CAG CCA CAG GCA CCA GGC GTG GAG GCC AGT GGG GCC GGG GAG     1169
Arg Asn Gln Pro Gln Ala Pro Gly Val Glu Ala Ser Gly Ala Gly Glu
345                 350                 355                 360

GCC CGG GCC AGC ACC GGG AGC TCA GAT TCT TCC CCT GGT GGC CAT GGG     1217
Ala Arg Ala Ser Thr Gly Ser Ser Asp Ser Ser Pro Gly Gly His Gly
                365                 370                 375

ACC CAG GTC AAT GTC ACC TGC ATC GTG AAC GTC TGT AGC AGC TCT GAC     1265
Thr Gln Val Asn Val Thr Cys Ile Val Asn Val Cys Ser Ser Ser Asp
            380                 385                 390

CAC AGC TCA CAG TGC TCC TCC CAA GCC AGC TCC ACA ATG GGA GAC ACA     1313
His Ser Ser Gln Cys Ser Ser Gln Ala Ser Ser Thr Met Gly Asp Thr
        395                 400                 405

GAT TCC AGC CCC TCG GAG TCC CCG AAG GAC GAG CAG GTC CCC TTC TCC     1361
Asp Ser Ser Pro Ser Glu Ser Pro Lys Asp Glu Gln Val Pro Phe Ser
    410                 415                 420

AAG GAG GAA TGT GCC TTT CGG TCA CAG CTG GAG ACG CCA GAG ACC CTG     1409
Lys Glu Glu Cys Ala Phe Arg Ser Gln Leu Glu Thr Pro Glu Thr Leu
425                 430                 435                 440

CTG GGG AGC ACC GAA GAG AAG CCC CTG CCC CTT GGA GTG CCT GAT GCT     1457
Leu Gly Ser Thr Glu Glu Lys Pro Leu Pro Leu Gly Val Pro Asp Ala
                445                 450                 455

GGG ATG AAG CCC AGT TAACCAGGCC GGTGTGGGCT GTGTCGTAGC CAAGGTGGGC     1512
Gly Met Lys Pro Ser
            460

TGAGCCCTGG CAGGATGACC CTGCGAAGGG GCCCTGGTCC TTCCAGGCCC CCACCACTAG   1572

GACTCTGAGG CTCTTTCTGG GCCAAGTTCC TCTAGTGCCC TCCACAGCCG CAGCCTCCCT   1632

CTGACCTGCA GGCCAAGAGC AGAGGCAGCG AGTTGGGGAA AGCCTCTGCT GCCATGGTGT   1692

GTCCCTCTCG GAAGGCTGGC TGGGCATGGA CGTTCGGGGC ATGCTGGGGC AAGTCCCTGA   1752

CTCTCTGTGA CCTGCCCCGC CCAGCTGCAC CTGCCAGCCT GGCTTCTGGA GCCCTTGGGT   1812

TTTTTGTTTG TTTGTTTGTT TGTTTGTTTG TTTCTCCCCC TGGGCTCTGC CCAGCTCTGG   1872

CTTCCAGAAA ACCCCAGCAT CCTTTTCTGC AGAGGGGCTT TCTGGAGAGG AGGGATGCTG   1932

CCTGAGTCAC CCATGAAGAC AGGACAGTGC TTCAGCCTGA GGCTGAGACT GCGGGATGGT   1992

CCTGGGGCTC TGTGTAGGGA GGAGGTGGCA GCCCTGTAGG GAACGGGGTC CTTCAAGTTA   2052

GCTCAGGAGG CTTGGAAAGC ATCACCTCAG GCCAGGTGCA GTGGCTCACG CCTATGATCC   2112
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-continued

CAGCACTTTG GGAGGCTGAG GCGGGTGGAT CACCTGAGGT TAGGAGTTCG AGACCAGCCT 2172

GGCCAACATG GTAAAACCCC ATCTCTACTA AAAATACAGA AATTAGCCGG GC 2224

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 461 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
  1               5                  10                  15

Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
             20                  25                  30

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
         35                  40                  45

Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
     50                  55                  60

Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
 65                  70                  75                  80

Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
                 85                  90                  95

Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
            100                 105                 110

Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
        115                 120                 125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
    130                 135                 140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145                 150                 155                 160

Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
                165                 170                 175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
            180                 185                 190

Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
        195                 200                 205

Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
    210                 215                 220

Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
225                 230                 235                 240

Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly
                245                 250                 255

Asp Phe Ala Leu Pro Val Gly Leu Ile Val Gly Val Thr Ala Leu Gly
            260                 265                 270

Leu Leu Ile Ile Gly Val Val Asn Cys Val Ile Met Thr Gln Val Lys
        275                 280                 285

Lys Lys Pro Leu Cys Leu Gln Arg Glu Ala Lys Val Pro His Leu Pro
    290                 295                 300

Ala Asp Lys Ala Arg Gly Thr Gln Gly Pro Glu Gln Gln His Leu Leu
305                 310                 315                 320

Ile Thr Ala Pro Ser Ser Ser Ser Ser Ser Leu Glu Ser Ser Ala Ser
                325                 330                 335
```

-continued

```
Ala Leu Asp Arg Arg Ala Pro Thr Arg Asn Gln Pro Gln Ala Pro Gly
            340                 345                 350

Val Glu Ala Ser Gly Ala Gly Glu Ala Arg Ala Ser Thr Gly Ser Ser
        355                 360                 365

Asp Ser Ser Pro Gly Gly His Gly Thr Gln Val Asn Val Thr Cys Ile
    370                 375                 380

Val Asn Val Cys Ser Ser Ser Asp His Ser Ser Gln Cys Ser Ser Gln
385                 390                 395                 400

Ala Ser Ser Thr Met Gly Asp Thr Asp Ser Ser Pro Ser Glu Ser Pro
                405                 410                 415

Lys Asp Glu Gln Val Pro Phe Ser Lys Glu Glu Cys Ala Phe Arg Ser
            420                 425                 430

Gln Leu Glu Thr Pro Glu Thr Leu Leu Gly Ser Thr Glu Glu Lys Pro
        435                 440                 445

Leu Pro Leu Gly Val Pro Asp Ala Gly Met Lys Pro Ser
    450                 455                 460
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 345 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..345

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
GTG AAA CTG CAG GAG TCT GGA CCT GAG CTG GTG AAG CCT GGG GCC TCA       48
Val Lys Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser
  1               5                  10                  15

GTG AAG ATT TCC TGC AAA ACT TCT GGC TTC GCA TTC AGT CAT TCT TGG       96
Val Lys Ile Ser Cys Lys Thr Ser Gly Phe Ala Phe Ser His Ser Trp
             20                  25                  30

ATG AAC TGG GTG AGG CAG AGG CCT GGA CAG GGT CTT GAA TGG ATT GGA      144
Met Asn Trp Val Arg Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
         35                  40                  45

CGG ATT TAT CCT GGA GAT GGA AAT ACT GAT TAC CCT GGG AAG TTC CAG      192
Arg Ile Tyr Pro Gly Asp Gly Asn Thr Asp Tyr Pro Gly Lys Phe Gln
     50                  55                  60

GGC CAG GCC ACA CTG ACT GCA GAC AAA TCT TCC AGC ACA GCC TAC ATG      240
Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met
 65                  70                  75                  80

CAA CTC TTC AGT CTG ACC TCT GTG GAC TCT GCG GTC TAT TTT TGT GCA      288
Gln Leu Phe Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Phe Cys Ala
                 85                  90                  95

CCC GGC CGT TGG TAC CTC GAA GTC TGG GGC CAA GGG ACC ACG GTC ACC      336
Pro Gly Arg Trp Tyr Leu Glu Val Trp Gly Gln Gly Thr Thr Val Thr
            100                 105                 110

GTC TCC TCA                                                          345
Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 amino acids
(B) TYPE: amino acid

-continued

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Val Lys Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser
  1               5                  10                  15

Val Lys Ile Ser Cys Lys Thr Ser Gly Phe Ala Phe Ser His Ser Trp
             20                  25                  30

Met Asn Trp Val Arg Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
         35                  40                  45

Arg Ile Tyr Pro Gly Asp Gly Asn Thr Asp Tyr Pro Gly Lys Phe Gln
     50                  55                  60

Gly Gln Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met
 65                  70                  75                  80

Gln Leu Phe Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Phe Cys Ala
                85                  90                  95

Pro Gly Arg Trp Tyr Leu Glu Val Trp Gly Gln Gly Thr Thr Val Thr
            100                 105                 110

Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 324 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..324

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
CCT GAG CTG GTG GCT CCT GGG GCC TCA GTG AAG ATT TCC TGC AAA GCT       48
Pro Glu Leu Val Ala Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala
  1               5                  10                  15

TCT GGC TAC GCA TTC AGT CAC TCT TGG ATG AAC TGG GTG AAG CAG AGG       96
Ser Gly Tyr Ala Phe Ser His Ser Trp Met Asn Trp Val Lys Gln Arg
             20                  25                  30

CCT GGA AAG GGT CTT GAG TGG ATT GGA CGG ATT CAT CCT GGA GAT GGA      144
Pro Gly Lys Gly Leu Glu Trp Ile Gly Arg Ile His Pro Gly Asp Gly
         35                  40                  45

GAC ACT GAC TAC AAT GGG AAC TTC AGG GGC AAG GCC ACA CTG ACT GCA      192
Asp Thr Asp Tyr Asn Gly Asn Phe Arg Gly Lys Ala Thr Leu Thr Ala
     50                  55                  60

GAC ACA TCC TCC AGC TCA GCC TAC ATG CAG CTC AGC AGC CTG ACC TCT      240
Asp Thr Ser Ser Ser Ser Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser
 65                  70                  75                  80

GTG GAT TCT GCG GTC TAC TTC TGT GCA CCC GGC CGT TGG TAC CTC GAG      288
Val Asp Ser Ala Val Tyr Phe Cys Ala Pro Gly Arg Trp Tyr Leu Glu
                85                  90                  95

GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC TCA                      324
Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Pro Glu Leu Val Ala Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala
 1               5                  10                  15

Ser Gly Tyr Ala Phe Ser His Ser Trp Met Asn Trp Val Lys Gln Arg
            20                  25                  30

Pro Gly Lys Gly Leu Glu Trp Ile Gly Arg Ile His Pro Gly Asp Gly
        35                  40                  45

Asp Thr Asp Tyr Asn Gly Asn Phe Arg Gly Lys Ala Thr Leu Thr Ala
    50                  55                  60

Asp Thr Ser Ser Ser Ser Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser
65                  70                  75                  80

Val Asp Ser Ala Val Tyr Phe Cys Ala Pro Gly Arg Trp Tyr Leu Glu
                85                  90                  95

Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 307 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..306

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
GTG TCC CTG CAG GAG TCT GGG GGA GGC TTA GTG CAG CCT GGA GGG TCC       48
Val Ser Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser
 1               5                  10                  15

CGG AAA CTC TCC TGT GCA GCT TCT GGA TTC ACT TTC AGT AGC TTT GGA       96
Arg Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly
            20                  25                  30

ATG CAC TGG GTT CGT CAG GCT CCA GAG AAG GGG CTG GAG TGG GTC GCA      144
Met His Trp Val Arg Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala
        35                  40                  45

TAC ATT AGT AGT GGC AGT AGT ACC CTC CAC TAT GCA GAC ACA GTG AAG      192
Tyr Ile Ser Ser Gly Ser Ser Thr Leu His Tyr Ala Asp Thr Val Lys
    50                  55                  60

GGC CGA TTC ACC ATC TCC AGA GAC AAT CCC AAG AAC ACG CTG TTC CTG      240
Gly Arg Phe Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu
65                  70                  75                  80

CAA ATG AAA CTA CCC TCA CTA TGC TAT GGA CTA CTG GGG CCA AGG GAC      288
Gln Met Lys Leu Pro Ser Leu Cys Tyr Gly Leu Leu Gly Pro Arg Asp
                85                  90                  95

CAC GGT CAC CGT CTC CTC A                                           307
His Gly His Arg Leu Leu
            100
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids

-continued

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Val Ser Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser
  1               5                  10                  15

Arg Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly
            20                  25                  30

Met His Trp Val Arg Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala
        35                  40                  45

Tyr Ile Ser Ser Gly Ser Ser Thr Leu His Tyr Ala Asp Thr Val Lys
    50                  55                  60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu
65                  70                  75                  80

Gln Met Lys Leu Pro Ser Leu Cys Tyr Gly Leu Leu Gly Pro Arg Asp
                85                  90                  95

His Gly His Arg Leu Leu
            100
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 358 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
TCC TCC CTG GCT ATG TCA GTA GGA CAG ATG GTC ACT ATG AGC TGC AAG      48
Ser Ser Leu Ala Met Ser Val Gly Gln Met Val Thr Met Ser Cys Lys
  1               5                  10                  15

TCC AGT CAG AGC CTT TTA ACT AGT AGC ACT CAA AAG AAC TCT TTG GCC      96
Ser Ser Gln Ser Leu Leu Thr Ser Ser Thr Gln Lys Asn Ser Leu Ala
            20                  25                  30

TGG TAC CAG CAG ACA CCA GGA CAG TCT CCT AAA CTT CTG ATA TAC TTT     144
Trp Tyr Gln Gln Thr Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Phe
        35                  40                  45

GCA TCC ACT AGG CTA TCT GGG GTC CCT GAT CGC TTC ATA GGC AGT GGA     192
Ala Ser Thr Arg Leu Ser Gly Val Pro Asp Arg Phe Ile Gly Ser Gly
    50                  55                  60

TCT GGG ACA GAT TTC ACT CTT ACC ATC AGC AGT GTG CAG GCT GAA GAC     240
Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp
65                  70                  75                  80

CTG GCA GAT TAC TTC TGT CAG CAA CAT TAT AGC ACT CCA TTT ACG TTC     288
Leu Ala Asp Tyr Phe Cys Gln Gln His Tyr Ser Thr Pro Phe Thr Phe
                85                  90                  95

GGC TCG GGG ACA AAG TTG GAA ATA GAG CGG GCT GAT GCT GCA CCA ACT     336
Gly Ser Gly Thr Lys Leu Glu Ile Glu Arg Ala Asp Ala Ala Pro Thr
            100                 105                 110

GTA TCC ATC TTC CCA CCA TCC A                                      358
Val Ser Ile Phe Pro Pro Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:11:

-continued

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Ser Ser Leu Ala Met Ser Val Gly Gln Met Val Thr Met Ser Cys Lys
  1               5                  10                  15

Ser Ser Gln Ser Leu Leu Thr Ser Ser Thr Gln Lys Asn Ser Leu Ala
             20                  25                  30

Trp Tyr Gln Gln Thr Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Phe
         35                  40                  45

Ala Ser Thr Arg Leu Ser Gly Val Pro Asp Arg Phe Ile Gly Ser Gly
     50                  55                  60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp
 65                  70                  75                  80

Leu Ala Asp Tyr Phe Cys Gln Gln His Tyr Ser Thr Pro Phe Thr Phe
                85                  90                  95

Gly Ser Gly Thr Lys Leu Glu Ile Glu Arg Ala Asp Ala Ala Pro Thr
            100                 105                 110

Val Ser Ile Phe Pro Pro Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 153 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1               5                  10                  15

Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly
            20                  25                  30

Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr
        35                  40                  45

Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg
    50                  55                  60

Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp
65                  70                  75                  80

Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu
                85                  90                  95

Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val
            100                 105                 110

His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala
        115                 120                 125

Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn Cys Lys
    130                 135                 140

Lys Ser Leu Glu Cys Thr Lys Leu Cys
145                 150
```

(2) INFORMATION FOR SEQ ID NO:13:

-continued

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 163 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys
1               5                  10                  15

Ser Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr
            20                  25                  30

Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu
        35                  40                  45

Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Asp
    50                  55                  60

Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys
65                  70                  75                  80

Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys
                85                  90                  95

Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala
            100                 105                 110

Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro
        115                 120                 125

Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His
    130                 135                 140

Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala
145                 150                 155                 160

Val Cys Thr
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Gln Asn Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro
1               5                  10                  15

Cys Pro Pro Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp
            20                  25                  30

Glu Pro Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys
        35                  40                  45

Ala His Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly
    50                  55                  60

His Gly Leu Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys
65                  70                  75                  80

Cys Arg Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His
                85                  90                  95

Cys Asp Pro Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr
            100                 105                 110

Leu Thr Ser Asn Thr Lys Cys
        115
```

-continued

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 159 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Ala Cys Pro Thr Gly Leu Tyr Thr His Ser Gly Glu Cys Cys Lys Ala
1               5                  10                  15

Cys Asn Leu Gly Glu Gly Val Ala Gln Pro Cys Gly Ala Asn Gln Thr
            20                  25                  30

Val Cys Glu Pro Cys Leu Asp Ser Val Thr Ser Ser Asp Val Val Ser
        35                  40                  45

Ala Thr Glu Pro Cys Lys Pro Cys Thr Glu Cys Val Gly Leu Gln Ser
    50                  55                  60

His Ser Ala Pro Cys Val Glu Ala Asp Asp Ala Val Cys Arg Cys Ala
65                  70                  75                  80

Tyr Gly Tyr Tyr Gln Asp Glu Thr Thr Gly Arg Cys Glu Ala Cys Arg
                85                  90                  95

Val Cys Glu Ala Gly Ser Gly Leu Val Phe Ser Cys Gln Asp Lys Gln
            100                 105                 110

Asn Thr Val Cys Glu Glu Cys Pro Asp Gly Thr Tyr Ser Asp Glu Ala
        115                 120                 125

Asn His Val Asp Pro Cys Leu Pro Cys Thr Val Cys Glu Asp Thr Glu
    130                 135                 140

Arg Gln Leu Arg Glu Cys Thr Arg Trp Ala Asp Ala Glu Cys Glu
145                 150                 155
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 162 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Ala Cys Arg Glu Lys Gln Tyr Leu Ile Asn Ser Gln Cys Cys Ser Leu
1               5                  10                  15

Cys Gln Pro Gly Gln Lys Leu Val Ser Asp Cys Thr Glu Phe Thr Glu
            20                  25                  30

Thr Glu Cys Leu Pro Cys Gly Glu Ser Glu Phe Leu Asp Thr Trp Asn
        35                  40                  45

Arg Glu Thr His Cys His Gln His Lys Tyr Cys Asp Pro Asn Leu Gly
    50                  55                  60

Leu Arg Val Gln Gln Lys Gly Thr Ser Glu Thr Asp Thr Ile Cys Thr
65                  70                  75                  80

Cys Glu Glu Gly Trp His Cys Thr Ser Glu Ala Cys Glu Ser Cys Val
                85                  90                  95

Leu His Arg Ser Cys Ser Pro Gly Phe Gly Val Lys Gln Ile Ala Thr
            100                 105                 110

Gly Val Ser Asp Thr Ile Cys Glu Pro Cys Pro Val Gly Phe Phe Ser
```

-continued

```
        115                 120                 125

Asn Val Ser Ser Ala Phe Glu Lys Cys His Pro Thr Ser Cys Glu Thr
    130                 135                 140

Lys Asp Leu Val Val Gln Gln Ala Gly Thr Asn Lys Thr Asp Val Val
145                 150                 155                 160

Cys Gly
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 140 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Asn Cys Val Lys Asp Thr Tyr Pro Ser Gly His Lys Cys Cys Arg Glu
1               5                   10                  15

Cys Gln Pro Gly His Gly Met Val Ser Arg Cys Asp His Thr Arg Asp
            20                  25                  30

Thr Val Cys His Pro Cys Glu Pro Gly Phe Tyr Asn Glu Ala Val Asn
        35                  40                  45

Tyr Asp Thr Cys Lys Gln Cys Thr Gln Cys Asn His Arg Ser Gly Ser
    50                  55                  60

Glu Leu Lys Gln Asn Cys Thr Pro Thr Glu Asp Thr Val Cys Gln Cys
65                  70                  75                  80

Arg Pro Gly Thr Gln Pro Arg Gln Asp Ser Ser His Lys Leu Gly Val
                85                  90                  95

Asp Cys Val Pro Cys Pro Pro Gly His Phe Ser Pro Gly Ser Asn Gln
            100                 105                 110

Ala Cys Lys Pro Trp Thr Asn Cys Thr Leu Ser Gly Lys Gln Ile Arg
        115                 120                 125

His Pro Ala Ser Asn Ser Leu Asp Thr Val Cys Glu
    130                 135                 140
```

What is claimed is:

1. An antibody to human TNF Binding Protein TBP-II (residues 27-214 of SEQ ID NO:2) which specifically recognizes said protein.

2. An antibody as claimed in claim 1 which is further characterized in that it blocks the binding of TNF to U937 and K562 cells.

3. An antibody as claimed is claim 1 further characterized in that it does not block the binding of TNF to HeLa and MCF7 cells.

4. An antibody according to claim 1 which is a polyclonal antibody.

5. An antibody according to claim 1 which is a monoclonal antibody.

6. A monoclonal antibody according to claim 5 produced from a hybridoma formed by fusion of myeloma cells with spleen cells and lymphocytes of mice previously immunized with TBP-II (residues 27-214 of SEQ ID NO:2).

7. A monoclonal antibody according to claim 6 produced from hybridoma TBP-II 13-12 deposited in CNCM under designation I-929.

8. A monoclonal antibody according to claim 6 produced from hybridoma TBP-II 70-2, deposited in CNCM under designation I-928.

9. A peptide or antibody, which peptide or antibody inhibits the signaling for the cytotoxic effect by the p75 TNF receptor but does not block TNF binding to the p75 TNF receptor, said peptide or antibody comprising the antigen binding portion of an antibody which binds to an extracellular domain of the C-terminal cysteine loop of the p75 TNF receptor, which loop consists of the amino acid sequence Cys-185 to Thr-201 of SEQ ID NO:3, with the proviso that said antigen binding portion is not that of a monoclonal antibody from clones 32 or 70, subcultures of which were deposited as CNCM No. I-1358 or CNCM No. I-928, respectively.

10. An antibody or a peptide which binds to TBP-II (residues 27–214 of SEQ ID NO:2), comprising a fraction of monoclonal antibody 70-2 (CNCM No. I-928), which fraction binds to TBP-II.

11. An antibody or peptide in accordance with claim 10, comprising monoclonal antibody 70-2 (CNCM No. I-928).

12. An antibody or a peptide which binds to TBP-II (residues 27–214 of SEQ ID NO:2), comprising a fraction of monoclonal antibody 57-1 (CNCM No. I-1696), which fraction binds to TBP-II.

13. An antibody or peptide in accordance with claim 12, comprising monoclonal antibody 57-1 (CNCM No. I-1696).

14. An antibody or a peptide which binds to TBP-II (residues 27–214 of SEQ ID NO:2), comprising a fraction of monoclonal antibody 32-5 (CNCM No. I-1358), which fraction binds to TBP-II.

15. An antibody or a peptide which binds to TBP-II (residues 27-214 of SEQ ID NO:2), comprising a fraction of an antibody which specifically recognizes TBP-II.

* * * * *

REVISED INTERIM WRITTEN DESCRIPTION GUIDELINES TRAINING MATERIALS

Contents

EXHIBIT C

REVISED INTERIM WRITTEN DESCRIPTION

TRAINING EXAMPLES

SYNOPSIS OF APPLICATION OF WRITTEN DESCRIPTION GUIDELINES

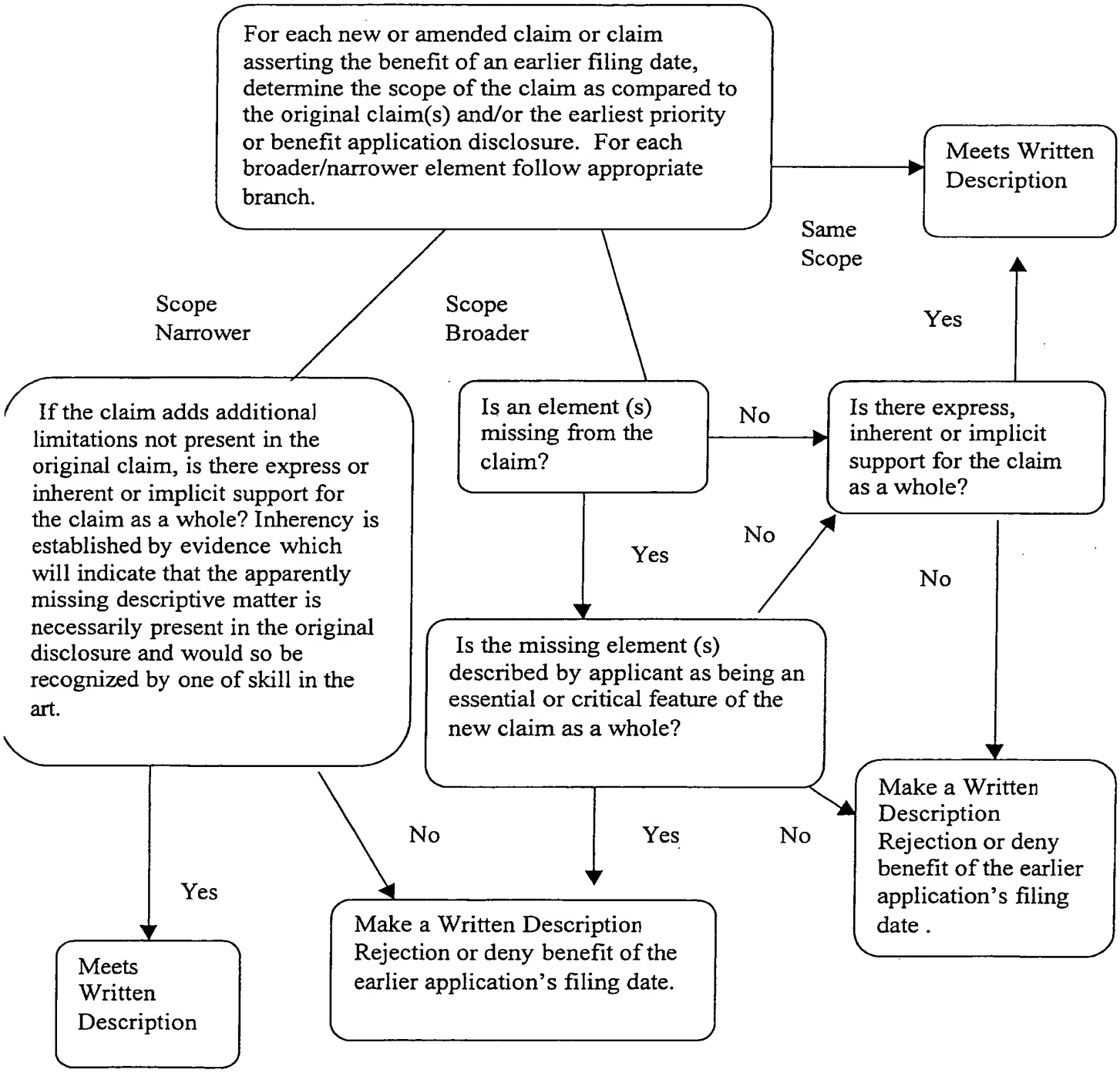
It is assumed at this point in the analysis that the specification has been reviewed and an appropriate search of the claimed subject matter has been conducted. It is also assumed that the examiner has identified which features of the claimed invention are conventional taking into account the body of existing prior art. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed. If the examiner determines that the application does not comply with the written description requirement, the examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. It should also be noted that the test for an adequate written description is separate and distinct from the test under the enablement criteria of 35 U.S.C. § 112 first paragraph. The absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. 112, para. 1, for lack of adequate written description. Limitations may not, however, be imported into the claims from the specification.

The following examples only describe how to determine whether the written description requirement of 35 U.S.C. 112, para. 1 is satisfied. Regardless of

the outcome of that determination, Office personnel must complete the patentability determination under all the relevant statutory provisions of Title 35 of the U.S. Code. Once Office personnel have concluded analysis of the claimed invention under all the statutory provisions, including 35 U.S.C. 101, 112, 102, and 103, they should review all the proposed rejections and their bases to confirm their correctness. Only then should any rejection be imposed in an Office action. The Office action should clearly communicate the findings, conclusions, and reasons which support them. When possible, the Office action should offer helpful suggestions on how to overcome rejections.

Written Description Amended or New Claims, or Claims Asserting the Benefit of an Earlier Filing Date

Decision Tree



Written Description
Original Claims

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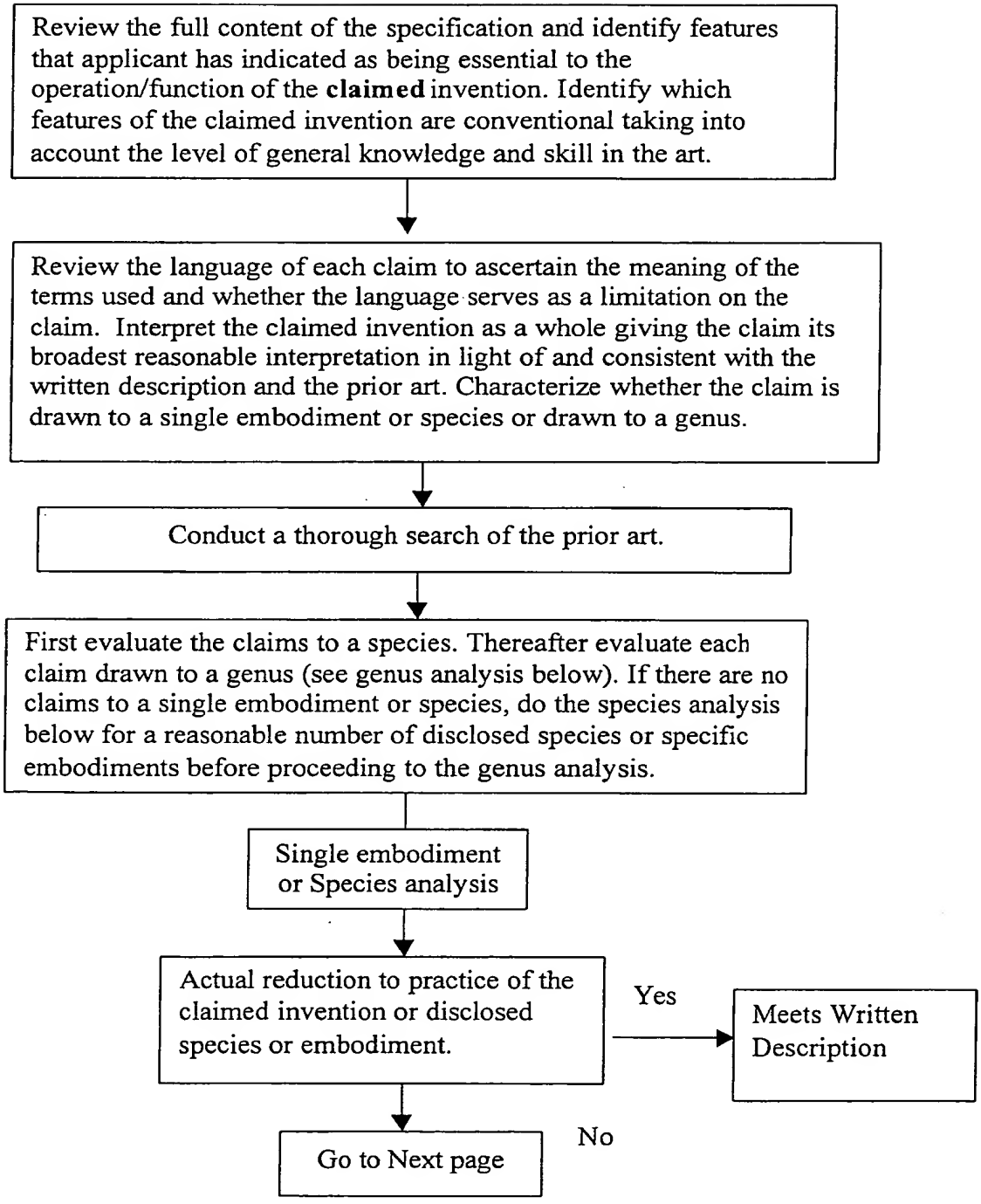

Written Description

Original Claims

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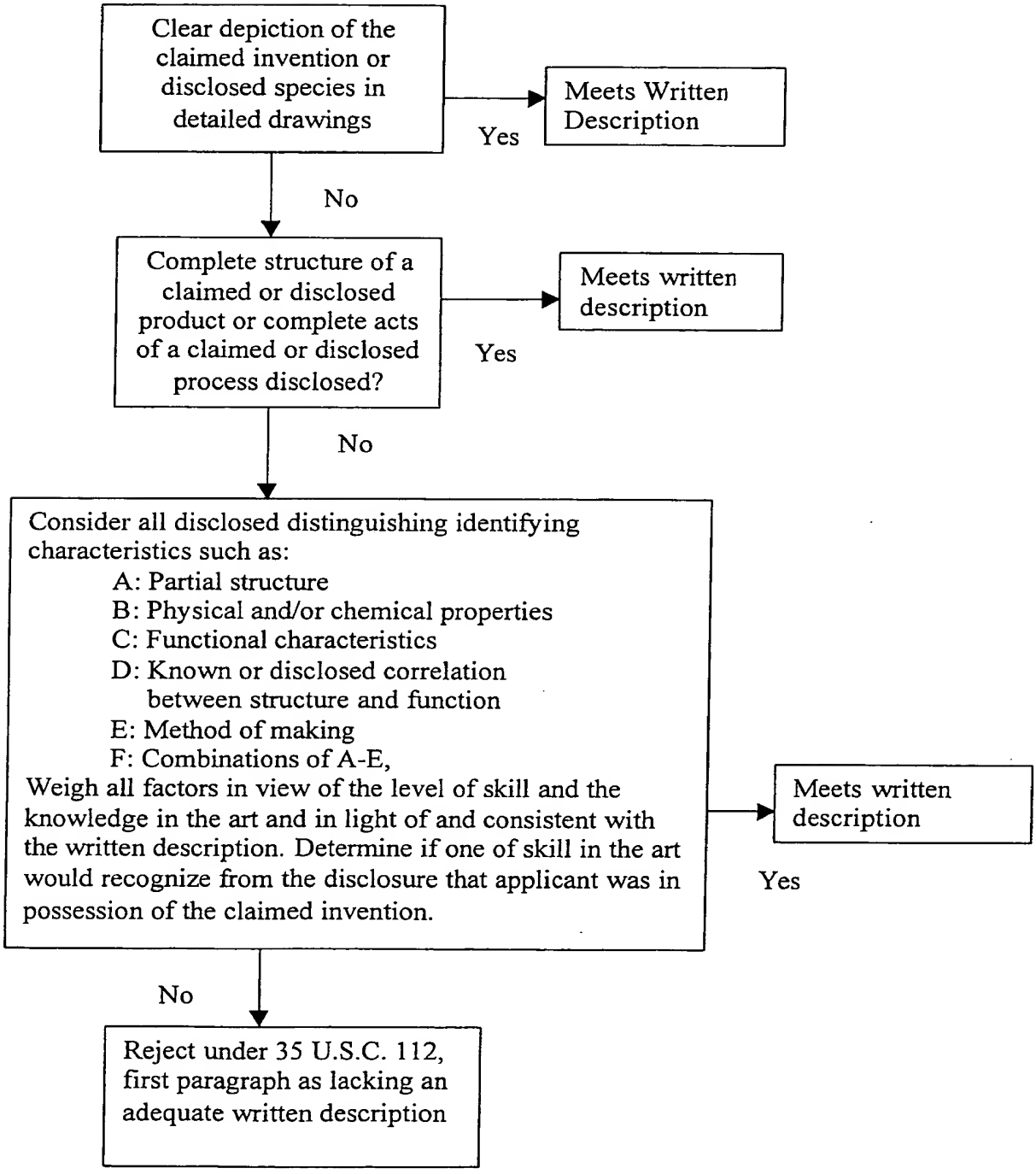

Written Description

Original Claims

Decision Tree

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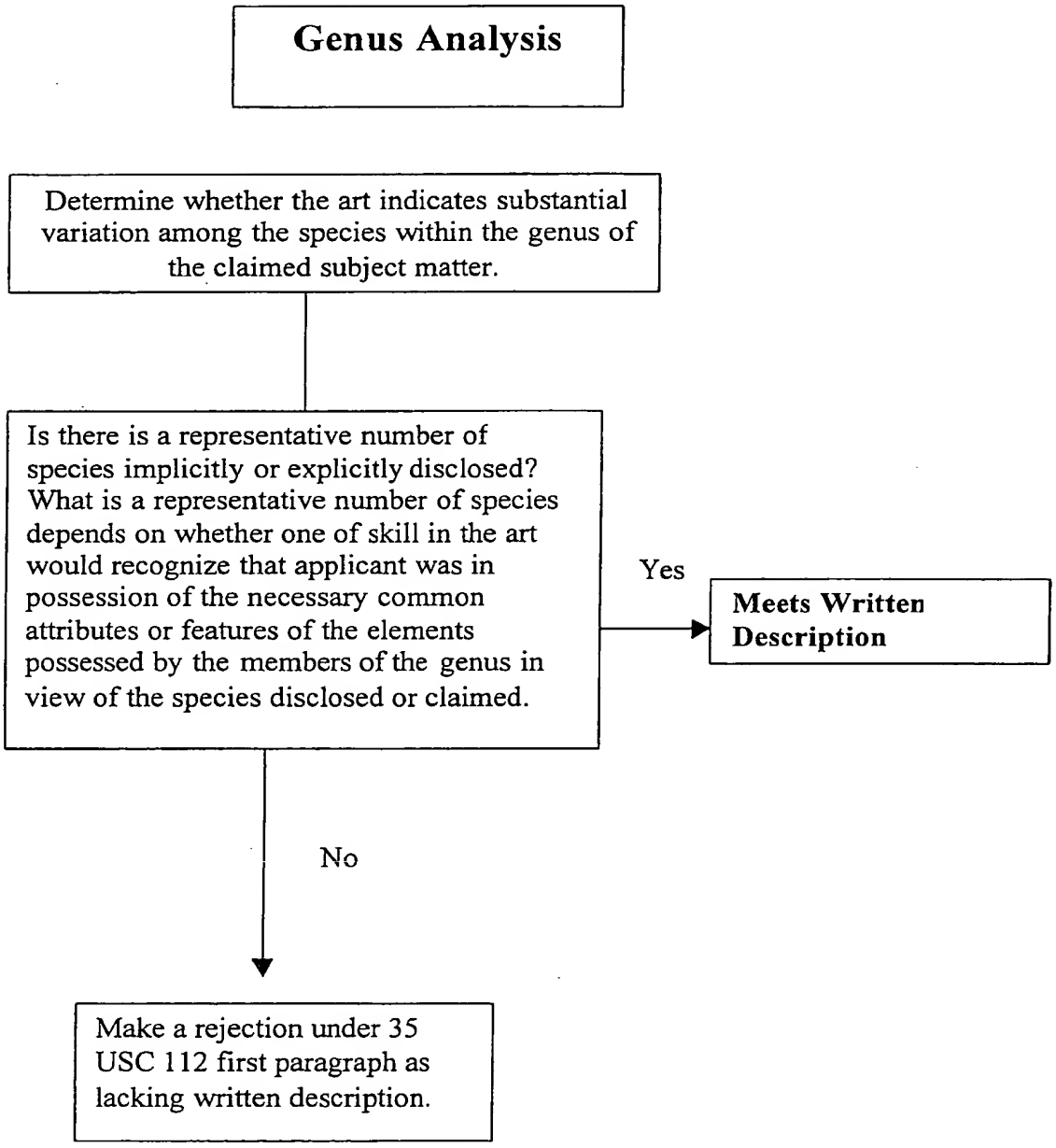

Example 16: Antibodies

Specification: The specification teaches that antigen X has been isolated and is useful for detection of HIV infections. The specification teaches antigen X as purified by gel filtration and provides characterization of the antigen as having a molecular weight of 55 KD. The specification also provides a clear protocol by which antigen X was isolated. The specification contemplates but does not teach in an example antibodies which specifically bind to antigen X and asserts that these antibodies can be used in immunoassays to detect HIV. The general knowledge in the art is such that antibodies are structurally well characterized. It is well known that all mammals produce antibodies and they exist in five isotypes, IgM, IgG, IgD, IgA and IgE. Antibodies contain an effector portion which is the constant region and a variable region that contains the antigen binding sites in the form of complementarity determining regions and the framework regions. The sequences of constant regions as well as the variable regions subgroups (framework regions) from a variety of species are known and published in the art. It is also well known that antibodies can be made against virtually any protein.

Claim: An isolated antibody capable of binding to antigen X.

Analysis:

A review of the full content of the specification indicates that antibodies which bind to antigen X are essential to the operation of the claimed invention. The level of skill and knowledge in the art of antibodies at the time of filing was such that production of antibodies against a well-

characterized antigen was conventional. This is a mature technology where the level of skill is high and advanced.

The claim is directed to any antibody which is capable of binding to antigen X.

A search of the prior art indicates that antigen X is novel and unobvious.

Considering the routine art-recognized method of making antibodies to fully characterized antigens, the well defined structural characteristics for the five classes of antibody, the functional characteristics of antibody binding, and the fact that the antibody technology is well developed and mature, one of skill in the art would have recognized that the spectrum of antibodies which bind to antigen X were implicitly disclosed as a result of the isolation of antigen X.

Conclusion: The disclosure meets the requirement under 35 USC 112 first paragraph as providing an adequate written description of the claimed invention.